(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 August 2006 (24.08.2006)

PCT

(10) International Publication Number
WO 2006/088385 A2

(51) International Patent Classification:
A61K 36/00 (2006.01)

(21) International Application Number:
PCT/RO2006/000003

(22) International Filing Date:
15 February 2006 (15.02.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
a 2005 00117 18 February 2005 (18.02.2005) RO

(71) Applicant *(for all designated States except US)*: **S.C. BIOTEHNOS S.A.** [—/RO]; 18, Dumbrava Rosie Street, R-020464 Bucharest 2 (RO).

(72) Inventors; and

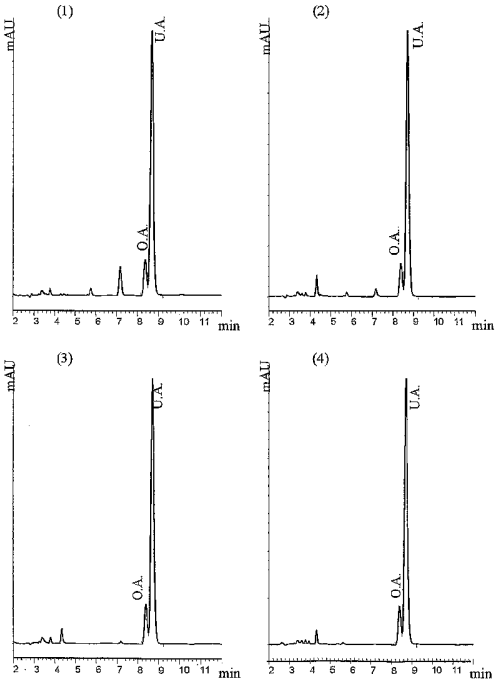
(75) Inventors/Applicants *(for US only)*: **TURCANU, Liudmila** [RO/RO]; 39, Drumul Taberei Street, Bloc OS 4, scara A, ap. 8, R-061359 Bucharest (RO). **MANZATU, Liliana** [RO/RO]; 49, Sandu Aldea Street, R-012062 Bucharest 1 (RO). **TARALUNGA, Gheorghe** [RO/RO]; 298, Calea Mosilor, bloc 48, scara 1, etaj 6, ap. 22, R-020898 Bucharest 2 (RO). **ZGLIMBEA, Lenuta** [RO/RO]; 8, Dreptatii Street, bloc O10, scara 1, etaj 7, ap. 48, R-060886 Bucharest 6 (RO). **TILICA, Maria** [RO/RO]; 10, Frumusani Street, bloc 94, etaj 2, ap. 18, R-041965 Bucharest 4 (RO). **NITA, Roxana, Andreea** [RO/RO]; 29 Pravat Street, bloc P7,, scara 7, etaj 4, ap. 140, R-061567 Bucharest 6 (RO).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: BIOACTIVE COMPLEX OF TRITERPENE ACIDS, ITS PRODUCTION PROCESS AND MEDICINAL PRODUCTS WITH THERAPEUTICAL USES



Chromatograms for bioactive complex of triterpene acids produced from: (1) Salvia Sp., (2) Lavandula Sp., (3) Sambucus Nigra, (4) Crataegus Sp.; O.A. = oleanolic acid; U.A. = ursolic acid.

(57) Abstract: The invention relates to a bioactive complex of triterpene acids, as such or in the form of Na, K, NH4 salts of minimum 90 % purity, with a standardized content formed of: minimum 75 % ursolic acid, 10...15 oleanolic acid and 4...10 % other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, produced from the plants *Salvia sp., Lavandula sp., Sambucus nigra* and *Crataegus sp.* The production process recommends increase of selectivity and efficiencies of extraction, purification and crystallization steps by using structured waters ("I" - acid pH water and "S" - alkaline pH water) in the composition of solvents used. The bioactive complex of triterpene acids has a marked and diversified pharmacological activity for bioregulating proteinic, glucidic and lipidic metabolism, particularly on aged organisms, as well as immunoprotective, antioxidizing, hepatoprotective, and retarding activity in tumour progression, being conditioned as new medicinal products for internal and/or external use with applicability in human and veterinary therapy.

WO 2006/088385 A2

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BIOACTIVE COMPLEX OF TRITERPENE ACIDS, ITS PRODUCTION PROCESS AND MEDICINAL PRODUCTS WITH THERAPEUTICAL USES

TECHNICAL FIELD

The present invention relates to a bioactive complex of triterpene acids, as such or in the form of Na, K, or NH_4 salts, to its production process from the plants *Salvia species, Lavandula species, Sambucus nigra* and *Crataegus species* and to medicinal products based on it, applicable in therapeutical areas of large social incidence, for both human and veterinary use.

The bioactive complex of triterpene acids produced according to the invention, has a high purity (at least 90%), being standardized in the content of triterpene acids and characterized by pharmacotoxicologic screening, in point of specific activity and safety in administration, which allows its use as a basic bioactive component of some medicinal products for internal or external use, in human and veterinary therapeutics.

BACKGROUND ART

Given the lately published pharmacological studies on triterpene acids (particularly the ursolic and oleanolic ones), these acids are known that, though isomers, show some structural differences that make a differentiation of pharmacological effects which, although similar to a large extent, they are not identical. That is why, especially when a mixture of triterpene acids is recommended to be used in therapeutics, it is highly important and essential to accurately know their proportions, since a content variability in the mixture may cause a variability in product pharmacological activity and bioavailability (D.BARICEVIC - Journal of Ethnopharmacology, 72(2001), 125-132; Paik KEE-JOO et al. - Arch.Pharmacology Research 1998, 21(4), 398-405, Chemical Abstracts vol. 129, 1998 (258259 b); RINGBORN Therese et al. – Journal of National Products, 1998, 61(10), 1212-1215, Chemical Abstracts, vol.129, 1998 (239627 d); Es-SAADY D. – Mediaters Inflammation 1994, 3(3), 181-184, Chemical Abstracts vol.121, 1994 (194953 y); A. NAJID et al. – Federation of European Biochemical Societies vol.299, no.3, 213-217, 1992).

Analysing patented processes regarding production of pure or mixed triterpene acids, most of them are found to be presented at laboratory level without showing purity for the product obtained, with specification of analytical method applied. In the latest years only, patents were published relating to production of some bioproducts based on mixed ursolic and oleanolic acids in the form of low purity (30...65%) concentrates,

analytically evaluated by HPLC or GC and intended to be used as nutritional supplements.

A general evaluation of lately patents and patent applications, that claim processes for producing pure or mixed triterpene acids (especially the ursolic and oleanolic ones), as such or in the form of salts, with inorganic or organic bases, leads to their classification in terms of the following criteria:

a) sources of plant raw materials:

- leaves and herba, harvested from plants of wild flora or cultures, whose processing results in obtaining some purified products with therapeutical applications;
- fruit residues, resulted as waste from food industry, by the processing of which concentrates with low content of triterpene acids (30...65%) are produced, utilizable to obtain some nutritional food supplements.

b) finished product making the main objective of the patented process:

- pure triterpene acids, as such or in the form of salts;
- mixture of triterpene acids, triglycerides, sugars etc. prevalently produced from fruit residues.

Further on, the latest patents and patent applications are presented, that relate to processes for producing triterpene acids (especially the ursolic and/or oleanoic ones);

- Patent GR 10011738/07.06.1993, entitled "Method for the separation of ursolic and oleanolic acids from leaves of Olea europaea", is a laboratory method that recommends extraction of dry leaves of Olea europaea with ethyl alcohol 95^{C}, at ambient temperature, for 15 days, precipitation of triterpene acids of alcohol extract by its dilution with water, sediment separation and drying, followed by its purification by successive extractions with petroleum ether. An analytical method by thin layer chromatography (TLC) is shown, by means of which the sediment purified with petroleum ether is qualitatively evaluated, as consisting of ursolic acid, and the petroleum ether extracts, concentrated up to wet residue (sicc), contain oleanolic acid.

The quantitative determination is not mentioned for ursolic and oleanolic acids separated as above, in order to be possible to estimate purity of substances and efficiency of separation process.

- Patent US 6740778/25.05.2004, entitled "Method for the preparation of oleanolic and/or maslinic acids", relates to a method for preparing these acids as such or in the form of physiologically acceptable salts, using Olea europaea plant as raw material from which the material remained after olive oil extraction, is processed.

Hydrophilic (alcohols, acetone) or lipophilic (hexane, chloroform, ethyl acetate) organic solvents are used as extraction solvents and the resulted extracts containing oleanolic or maslinic acid are purified by liquid-liquid extraction or silica gel column chromatography.

Triterpene acids produced in this way are mentioned to have a purity of 85...100%, and the purity of salts thereof is minimum 90%.

Although the method leads to production of some high purity substances, it has the disadvantage of some technological flows hard to be industrially applied, by both the recommended methods for purification and multitude of organic solvents used.

- Patent SU 18186346/3/27.09.1995, entitled "Production method for ursolic acid" relates to a method for preparing ursolic acid from fruit residues of *Oxycoccus quadripetelus* G, by isopropyl alcohol extraction, at ambient temperature, for 24 hours, vacuum concentration of alcohol extract up to residue, that is purified by petroleum ether extractions and successive changes into Na salt and purified ursolic acid.

An increase in processing efficiency and quality of substance produced is mentioned, without specifying its purity, too.

- Patent RU 2108107/10.04.1998, entitled: "Production method for a biologically active total content of triterpene acids salts", relates to a method for preparing triterpene acids from white fir tree (*Abies sp.)* species, in the form of a total content of Na salts of triterpene acids, that recomands plant raw material extraction with a petroleum ether – ethyl acetate mixture, extractive solutions concentration up to residue, which is dissolved in ethyl acetate, and the organic solution is alkalinized with 2% NaOH solution at pH=9, afterwards the alkaline aqueous extract, with pH=7...8, is vacuum concentrated, until producing a wet extract (sicc) in the form of powder, constituting the total content of triterpene acids as Na salt, of 38...45%.

Chemical structure and denomination of triterpene acids produced according to the patented process is not specified but, from literature data, the coniferous varieties are known to contain triterpene compounds with other chemical structures than those of ursolic and oleanolic acids.

- Patent RU 2108803/20.04.1998, entitled: "Production method for a biologically active total content of triterpene acids", relates to a method for preparing a total content of triterpene acids from fir tree needles, by methyl-butyl ether extraction, organic extract treatment with alkaline agent (2% NaOH aqueous solution), separation of alkaline aqueous solution, its acid treatment with 10% HCl solution, at pH=2 and isolation of finished product from acid solution by methyl-butyl ether extraction.

Like in the previous patent, denomination and chemical structure of triterpene acids isolated as above are not specified, only the resulted substance quantity being mentioned, without showing its purity, too.

- Patent RU 2151139/20.06.2000, entitled: "Method for the preparation of a biologically active total content of triterpene acids" relates to a method for extracting triterpene acids from fresh white fir tree needles or bark, steam treated beforehand, then extracted with organic solvents (methyl-buthyl ether; mixture: benzene – ethyl acetate; mixture: petroleum ether – ethyl acetate), followed by separation of the total content of triterpene acids by successive treatment of organic extract with alkaline and acid aqueous solutions.

The quantity of the product obtained is mentioned, without specifying chemical denomination of triterpene acids and product purity.

In addition, all the three above mentioned patents of Russian Federation, although they relate to the production of a biologically active total content of triterpene acids, show no pharmacological tests to evidence biological activity of the products obtained, on the basis of which the application possibilities in therapeutics could be evaluated.

A group of patents belonging to UNILEVER company presents different production methods for some fruit residues concentrates containing a mixture of ursolic, oleanolic acids and other components, being intended to be used as nutritional supplements.

- Patent EP 1161879/12.12.2001 and patent application US 2002037882/28.03.2002, relates to two laboratory and pilot processes – that recommend acetone reflux extraction (50...58^{0}C) of dry apple peels, extractive solution concentration up to residue and its crystallization from 50/50 acetone – water mixture up to 95/5 (laboratory process) or from hexane (pilot process).

A mixture of ursolic and oleanolic acids is obtained in variable proportions, that also contains other polar and non-polar components typical of apple peels. A conditioning process is also shown for triterpene mixture by association with mono-, di-, triglycerides and with oils containing unsaturated fatty acids, with a view to being used as nutritional supplements.

- Patent EP 1250852/23.10.2002 and US patent application 2003049365/13.03.2003, relates to a laboratory process for production of some triterpene acids concentrates (ursolic and oleanolic), as such or in the form of salts, that recommends processing of apple peels treated beforehand with acid aqueous solutions

(H_3PO_4) at pH=2 and with alkaline aqueous solutions (Na_2CO_3) at pH=8-12, followed by Soxhlet acetone extraction and extractive solution concentration up to wet residue (sicc) constituting the ursolic and oleanolic acid concentrate with a content of 30...65% in a 4:6 ratio, beside other components: sugars, glycerides and other triterpene compounds.

The use of such concentrates for the production of some nutritional supplements in the form of capsules or other conditioning forms, is mentioned.

- Patent CN 1358733/17.07.2002, relates to a process for extracting ursolic acid from *Ligustrum lucidum* leaves, that includes the following phases: leaves counter-flow extraction with reflux ethyl alcohol, extract concentration up to residue, that is washed with water, dried and the dry extract is dissolved in ethyl alcohol and decolourized, then concentrated and diluted in water, a precipitate being resulted; suspension pH is adjusted to 2...2.5, after then precipitate is separated, washed with neutral pH water and vacuum dried.

A crude extract is mentioned to be produced, having ursolic acid as a main component, without indicating product quantitative determination and purity, as well as the other existing compounds beside ursolic acid.

From the above mentioned data on the state of the art in producing triterpene acids from different sources of plant raw materials, one can conclude the following:

- most of processes for production of pure ursolic and oleanolic acids, make no mention of substance purity and its evaluation by quantitative analytical methods (HPLC, GC, a.o).
- the processes claiming production of some triterpene acids concentrates from fruit residues, based on a mixture of ursolic and oleanolic acids, though show their quantitative determination by HPLC, have the disadvantage of producing some low content products (30...65%) that are not applicable in therapeutics, conditioned as medicinal drugs, but only as nutritional supplements;
- in addition, fruit residues are known to have a low content in triterpene acids (< 0.5%) which negatively influences profitability of industrial-scale technologies in comparison with processing technologies for usual plant raw materials (medicinal and aromatic herbs);
- no processes are known for producing triterpene acid complex from the plants *Sambucus nigra* and *Crataegus species*;
- neither of the processes shown has technological data similar to the production process making the subject matter of the present invention nor pharmacological data on the specific biological activity of the claimed mixture

of triterpene acids, with a view to estimating the fields of therapeutical applicability.

The analysis of the state of the art in the field of triterpene acids pharmacology reveals that most of their pharmacological effects were demonstrated by scientific experimental researches whose results were published as articles or made the subject matter of some patent applications and patents.

Thus, J.Liu in Journal of Ethnopharmacology (49 (1995), 57-68), shows the following pharmacological effects revealed in oleanolic and ursolic acids: antiinflammatory, hepatoprotective, antihyperlipidemic, antiulcerous, hypoglycemic, antitumorigenic, antiHIV, antimicrobial.

Somova L.I., Shode F.O. et al. in Journal of Ethnopharmacology (2003 Feb. 84(2-3):299-305) and in International Journal of Phytotherapy & Phytopharm (3/1/2003), emphasize cardiovascular, antihypertensive, antihyperlipidemic, antiatherosclerotic and antioxidating activity of oleanolic and ursolic acids isolated from Olea europeea.

And Kuttan G. et al. in International Journal of Phytotherapy & Phytopharm (7/1/2003) show immunomodulating activity of ursolic, oleanolic and glycyrrhizic acids.

Patent US 4752606/1988 relates to a pharmaceutical composition based on oleanolic acid, as such or as physiologically acceptable salts, conditioned as tablets and utilizable for prophylactic or curative treatment of different ulcer disfunctions in the stomach or bowels.

Patent application US 10/488682/2002 published with no. US 2004/0235785 A1 relates to a pharmaceutical composition utilizable as dietetic supplement in cancer treatment or prevention, having the role of potentiator of antitumorigenic agents, used in immunotherapy and oncology. According to the invention, composition relies on compounds of terpene class (mono-, di-, tri-, sequiterpene).

Patent application JP 05-211065 published under no.07-048260/1995 relates to a bioproduct utilizable as a growing agent for blood cells (erythrocytes) based on ursolic acid, administered in daily doses of 1-1000 mg and having an effect of life prolonging in the X-ray treatment of different cancer forms.

Patent applications EP 1495754 A2 published on 12.01.2005 and EP 0943620 A2 published on 22.09.1999, relate to a method and a composition based on derivatives of betulinic acid applicable to prevent or inhibit growth of malignant tumours. A method for producing betulinic acid from the stem bark of *Ziziphus mauritiana* (Rhamnacae family), " in vitro" tests on cytotoxicity of betulinic acid as against different cancerigenic

cell lines (melanoma, fibrosarcoma, breast and colon cancer, epidermoid carcinoma) are shown, and results of "in vivo" tests in mouse, as well, confirming the quality of betulinic acid as antineoplastic agent.

Patent US 5985924/1999 relates to a new suppressing agent for metastases, with a low toxicity degree, based on ursolic acid, as such or in the form of salts, that can be orally or injection administered, in the postoperative treatment of various cancer forms.

Patent application JP 63166195 published under no.02017121 A/1990 relates to a medicinal drug for external use, utilizable to prevent skin epithelial cells to grow cancerigenic, based on ursolic or oleanolic acid, as such or mixtured.

Patent application JP 09-199323 published under no.JP 11-029467/1999 relates to a product having inhibitory effects on protease, based on ursolic acid or salts thereof, applicable for the treatment or prevention of different skin disfunctions (dermatitides) and conditioned in the form of ointment, cream, lotion and emulsion. Dermatoprotective effects of product are specified: antioxidating, hydrating and epidermis whitening.

Patent US 4857554/1989 relates to a method for the treatment of psoriasis based on daily use of an ointment containing ursolic and oleanolic acids in a 3:1 ratio, dispersed in a fatty base of lanoline and vaseline. Product action is shown by a clinical trial for 3 weeks, which revealed that severe forms of psoriasis were cured in a 60...70% percentage.

From data on the state of the art in the field of triterpene acids pharmacology, the following are concluded:

- all pharmacological studies on the activity specific to these acids were conducted separately for each acid and a multitude of pharmacological effects became evident which, although similar to a large extent, are not identical;

- neither of foregoing studies presents pharmacological tests performed on mixture of triterpene acids, in order to reveal synergism and potentiation of pharmacological effects that can be higher than those of each component used alone;

- preclinical or clinical pharmacological trials described within foregoing studies were not comparatively conducted on young, adult or aged organisms, although the fact is known that biochemical and hematological parameters differ considerably, depending on age that also influences therapeutical efficiency of the medicinal drug used.

The technical problem solved by the present invention is the production of a bioactive complex of triterpene acids containing ursolic, oleanolic acids and other triterpene acids consisting of one or more of the following acids: hydroxyursolic,

hydroxyoleanolic, betulinic, dehydrobetulilnic, as such or in the form of Na, K or NH_4 salts, by an industrially applicable technological process that carries out extraction of all structural types of triterpene acids existing in plant raw materials, and that, for increasing selectivity and efficiencies of processes for extraction, purification and crystallization, uses structured waters ("I" structured water with acid pH and "S" structured water with alkaline pH) in composition of solvents used to perform these technological stages, which lead to a finished product of a high purity (minimum 90%) and a standardized composition.

DISCLOSURE OF INVENTION

Bioactive complex of triterpene acids consists in that it has a minimum 90% content, represented by: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids such as: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic obtained in the form of free acids or as Na, K, NH_4 salts, by extraction from the following plant species: *Salvia species, Lavandula species, Sambucus nigra* and *Crataegus species*, showing the following pharmacological effects:

- bioregulating activity on proteinic, glucidic and lipidic metabolism, particularly on aged organisms, with applications in the preventive and curative therapy of metabolic and cardiovascular diseases of atherosclerotic etiology, especially in geriatrics;
- immunoprotective and hepatoprotective activity of counteracting immuno-suppressive and hepatotoxic adverse effects occurred after treatment with glucocorticosteroids and cytostatics;
- retarding activity in tumour progression;
- antioxidizing activity;
- local antiinflammatory activity.

The medicinal products, according to the invention, which contain as pharmaceutically active substance the bioactive complex of triterpene acids as such or in the form of salts, are conditioned as: injectable solution, perfusable emulsion, buvable solution, tablets, hard and/or soft jelly , eyewash, ophthalmic ointment, gel, cream, ointment, solution for external use or spray, suppositories, ovules, being applicable in human and veterinary therapy.

According to the production process for bioactive complex of triterpene acids from the present invention, plant raw materials, consisting of *Salvia species herba, Lavandula species herba, Sambucus nigra* – flowers or leaves or *Crataegus species herba*, are extracted by dynamic maceration in the 1/15...1/30 m/v ratio, plant

mass/solvent, with one of the extraction solvents represented by mixtures consisting of: 5...15% "I" structured water with 85....95% acetone or with 85...95% ethyl alcohol 95^C, for a period of 8...24h, at 15...80^0C, the resulted extractive solution being then purified by passage on a chromatographic column with acid active granulated carbon, next it is vacuum concentrated in a 1:10...1:20 v/v ratio of the initial volume; the resulted suspension is vacuum filtered, the after drying precipitate constituting the crude bioactive complex of triterpene acids with a minimum 70% content. For purification, this is reflux dissolved in a 1/100...1/200 m/v ratio in one of the solvents represented by mixtures consisting of: 5...10% "I" or "S" structured water with 90...95% ethyl alcohol 95^C or with 90...95% acetone. The resulted solution is purified by adsorption on acid active carbon, afterwards it is vacuum concentrated up to 1/10...1/20 of the initial volume, and the microcrystalline suspension is vacuum filtered, the after drying substance constituting the crystallized bioactive complex of triterpene acids, produced by 1...2 successive crystallizations, depending on the type of plant raw material processed, at the same parameters, excepting the second concentration carried out for 1/5 of the initial volume, with a minimum 90% content standardized in the following components: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.

To produce the complex of triterpene acids in the form of Na, K, or NH_4 salts, the crystallized product, with a minimum 90% content is dissolved in the 1/100...1/200 m/v ratio in the mixture of solvents with alkaline pH: 90...95% ethyl alcohol 95^C with 5...10% "S" structured water, that also contains 1% NaOH or 1% KOH or 5% NH_4OH 25%, followed by concentration of resulted solution and crystallization of bioactive complex of triterpene acids as Na, K or NH_4 salt, with a minimum 90% content.

Advantages of the invention, as compared with the state of the art, are the following:

- displays an industrially applicable technological process, for production of a bioactive complex of triterpene acids, as such or in the form of Na, K or NH_4 salts, with a high purity (minimum 90%), standardized and quantitatively determined by spectrophotometric method and HPLC for the content of ursolic, oleanolic acids and other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.
- recommends extraction of plant raw materials with mixtures of acid pH solvents, containing "I" structured water with pH=1.8...2.6, making a transfer of substances

through plant cell membrane higher to distilled water, which leads to a 10...15% increase of extraction efficiency;

- use of "I" structured water in the composition of extraction solvents results in a high degree of selectivity in the extraction process, since some of the chlorophyll existing in the plant remains precipitated in the plant tissue by acid pH and the extractive solution contains a lower quantity of ballast substances, which makes its subsequent processing to be carried out with higher efficiencies;

- purification of crude bioactive complex of triterpene acids and crystallization of purified product are also carried out in mixtures of acid pH solvents, containing "I" structured water, which ensures a selective crystallization and a high purity of finished product (minimum 90%) and a standardization of its content in components, as well;

- production of bioactive complex of triterpene acids in the form of Na, K or NH_4 salts is carried out in hydroalcoholic medium, containing "S" structured water, with pH=10...12, which determines selectivity of the crystallization process for the respective salts and their production with a high purity (of minimum 90%) and a phase efficiency of 95%;

- the technological process making the subject matter of the invention utilizes, concomitantly with ursolic and oleanolic acids existing in most plant raw materials as components in a majority, and other triterpene acids too, present in extractive solutions, as the following ones: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, that increase bioavailability of the entire complex, by step absorption, due to the difference in polarity and solubility of chemical structures of these acids.

- "in vivo" preclinical pharmacological trials performed on bioactive complex of triterpene acids, produced according to the invention, showed that it has a pharmacological activity higher to that of each component separately, due to some synergetic effects of reciprocal potentiation, applicable in production of new medicinal drugs.

The bioactive complex of triterpene acids, produced according to the invention, has a minimum 90% content, being standardized in the following components: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, having a considerable and diversified pharmacological activity, exerted on some vital functions, namely:

- bioregulation of proteinic, glucidic and lipidic metabolism, especially on aged organisms, with new applications in preventive and curative therapy of metabolic and cardiovascular diseases of atherosclerotic etiology, particularly in geriatric therapy;

- immunoprotective and hepatoprotective activity for counteracting adverse immunosuppressive and hepatotoxic effects, occurred after treatment with glucocorticosteroids, cytostatics, antiviral products and radiotherapy;

- retarding activity on tumour progression;

- local antiinflammatory activity.

Based on these pharmacological effects, the bioactive complex of triterpene acids, produced according to the invention, was conditioned as new medicinal products for human and/or veterinary internal use, such as: capsules, tablets, injectable solutions, buvable solutions, and for external use: eye washes, spray, gel, cream, ointment, suppositories, ovules, intended for new therapeutic applications, as compared to the already known ones.

The production process, according to the invention, consists in that the dried and pulverized plant raw material, with 2...4% triterpene acids, consisting of *Salvia species herba, Lavandula species herba* – not only the herba plant but also the residue resulted from producing essential oil by steaming, *Sambucus nigra* – flowers or leaves or *Crataegus species herba*, is extracted by dynamic maceration in the 1/15...1/30 m/v ratio, plant mass/solvent, with one of the extraction solvents represented by mixtures consisting of: 5...15% "I" structured water with 85...95% acetone or with 85...95% ethyl alcohol 95^{C}, for 8...24 h, at 15...80^{0}C, and the resulted extractive solution, in order to be purified, is passed on a chromatographic column with active granulated carbon with acid pH, then it is vacuum concentrated, at temperature of 35...40^{0}C, up to 1/10...1/20 of the initial volume, a suspension being obtained, that is kept for 12...24 h at 5...10^{0}C, next it is vacuum filtered, the obtained substance after drying at 105^{0}C, for 3 h, making the crude bioactive complex of triterpene acids with a minimum 70% content. For purification, the crude product is reflux dissolved at 80^{0}C in the 1/100...1/200 m/v ratio in one of the solvents represented by mixtures consisting of: 5...10% "I" or "S" structured water with 90...95% ethyl alcohol 95^{C} or with 90...95% acetone.

The obtained solution is purified by adsorption on acid pH active carbon, added in a 0.1...0.3% m/v ratio, is filtered, vacuum concentrated at 35...40^{0}C up to 1/10...1/20 of the initial volume, the resulted microcrystalline suspension is kept for 12...24 h at 5...10^{0}C for finishing crystallization, then it is vacuum filtered, the obtained precipitate is washed with 0.3...0.5 l distilled water up to neutral pH, is dried at 105^{0}C for at least 3 h

and pulverized, resulting in crystallized bioactive complex of triterpene acids, obtained through 1...2 successive crystallizations at the same parameters, depending on the type of plant raw material processed, excepting the second concentration made for 1/5 of the initial volume, with a minimum 90% standardized content, composed of the following components: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.

To produce the complex of triterpene acids in the form of Na, K or NH_4 salts, the crystallized product, with a minimum 90% content, is reflux dissolved at 80^0C in a 1/100...1/200 m/v ratio in the alkaline pH solvent mixture: 90...95% ethyl alcohol 95^C with 5...10% "S" structured water, that contains 1% NaOH or 1% KOH or 5% NH_4OH 25%; the obtained solution undergoes vacuum concentration at 35...40^0C up to 1/5...1/10 of the initial volume, the resulted suspension is kept for 12...24 h at 5...10^0C for finishing crystallization, followed by vacuum filtration, the precipitate is washed with 0.1...0.3 l distilled water up to neutral pH, dried at 105^0C for 3 h and pulverized. The crystallized bioactive complex of triterpene acids is produced, as Na, K or NH_4 salt, with minimum 90% estimated content, and a 95% phase efficiency.

MODES OF CARRYING OUT THE INVENTION

Further on, examples of carrying out the invention are described, that relate to the production process for bioactive complex of triterpene acids, to pharmacological tests performed for evidencing its specific activity and evaluating its applications in therapeutics, and to conditioned medicinal products as well.

Example no.1. – Variant A

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS, AT INDUSTRIAL SCALE, FROM *HERBA SALVIA SPECIES (SAGE)*

30 kg dried and pulverized plant raw material consisting of *herba Salvia species (Salvia officinalis, Salvia lavandulifolia, Salvia triloba, Salvia sclarea* etc.), with a content of 3...4% triterpene acids, are extracted with 500 l extraction solvent consisting of: 90 volumes (450 l) acetone + 10 volumes (50 l) "I" structured water, with pH=1.8...2.6, by dynamic maceration, for 12 h, at ambient temperature (15...30^0C), afterwards minimum 400 l extractive solution are collected, then passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution which is vacuum concentrated at 35...40^0C up to the 40 l volume, a suspension being obtained which is kept for 24 h at 5...10^0C, next it is vacuum filtered and the filter precipitate is dried at 105^0C, for 3 h, and pulverized. Minimum 1200 g slightly greenish yellow powder

are obtained, constituting the crude bioactive complex of triterpene acids with a minimum 70% content.

For the purification of product, an acid pH solvent mixture is used, consisting of 90 volumes (108 l) ethyl alcohol 95^{C} + 10 volumes (12 l) "I" structured water, with pH=1.8...2.6, where 1200 g powder, consisting of crude bioactive complex of triterpene acids (100 ml solvent mixture being calculated for 1 g substance), are added for dissolution; suspension is heated at 80^{0}C for 1 h until full dissolution of substance, and the resulted yellow solution is treated with 0.1...0.3% m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated at 35...40^{0}C up to 1/10 of the initial volume (12 l) when a microcrystalline suspension is produced, which is kept for 24 h at 5...10^{0}C for finishing crystallization, then it is vacuum filtered, the precipitate is washed with 0.5 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 630 g microcrystalline, white powder are resulted, composed of crystallized bioactive complex of triterpene acids, with a 96.6% content formed of: 80.9% ursolic acid, 10.2% oleanolic acid and 5.5% other triterpene acids, consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic which represents a final efficiency of at least 50% compared with 4% triterpene acids content of the processed plant raw material. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Variant B

30 kg dried and pulverized plant raw material, consisting *of herba Salvia species (Salvia officinalis, Salvia lavandulifolia, Salvia triloba, Salvia sclarea* etc.) with a 3...4% content of triterpene acids, are extracted with 500 l extraction solvent consisting of: 90 volumes (450 l) ethyl alcohol 95^{C} + 10 volumes (50 l) "I" structured water (pH=1.8...2.6) by dynamic maceration, for 12 h at ambient temperature (15...30^{0}C), then minimum 400 l extractive solution are collected, passed on a chomatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated at 35...40^{0}C, up to the 40 l volume, a suspension being obtained which is kept at 5...10^{0}C for 24 h, afterwards vacuum filtered, and the filter precipitate is dried at 105^{0}C for 3 h and pulverized.

Minimum 1200 g slightly greenish yellow powder are obtained, constituting the crude complex of triterpene acids, with a minimum 70% content.

To purify the crude product, the acid pH solvent mixture is used, composed of 90 volumes (108 l) acetone + 10 volumes (12 l) "I" structured water with pH=1.8...2.6, where 1200 g powder are added for dissolution, consisting of crude complex of triterpene acids (by calculating 100 ml solvent mixture for 1 g substance); suspension is heated at 80^{0}C for 1 h, up to full substance dissolution and the resulted yellow solution is treated with 0.1...0.3 m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated, at 35...40^{0}C, up to 1/10 of the initial volume (12 l), when a microcrystalline suspension is resulted which is kept for 24 h at 5...10^{0}C for finishing crystallization, then it is vacuum filtered, the precipitate is washed with 0.5 l distilled water, up to neutral pH, dried at 105^{0}C for 3h and then pulverized.

Minimum 640 g microcrystalline, white powder are resulted composed of crystallized bioactive complex of triterpene acids, with a 96.7% content, consisting of: 80.4% ursolic acid, 11.7 oleanolic acid and 4.6% other triterpene acids formed of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a final efficiency of minimum 50% compared with the 4% content of triterpene acids of the processed plant raw material.

This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.2 – Variant A

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS AT INDUSTRIAL SCALE, FROM *HERBA LAVANDULA SPECIES (LAVANDER)*

30 kg dried and pulverized plant raw material, consisting of *herba Lavandula species* (*Lavandula angustifolia, Lavandula vera, Lavandula spica, Lavandula fragrans, Lavandula latifolia,* etc.) or of lavender residue resulted from producing essential oil by steaming, with a 2...4% content of triterpene acids, are extracted with 500 l extraction solvent, consisting of: 90 volumes (450 l) acetone + 10 volumes (50 l) "I" structured water, with pH=1.8...2.6, by dynamic maceration, for 12 h, at ambient temperature (15...30^{0}C), then minimum 400 l extractive solution are collected which are passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution which is vacuum concentrated at 35...40^{0}C, up to a 40 l volume, a suspension being obtained which is kept at 5...10^{0}C for 24 h, then vacuum filtered, and the filter precipitate is dried at 105^{0}C for 3 h and pulverized. Minimum 900 greenish powder are produced which constitute the crude complex of triterpene acids, with a minimum 70% content.

To purify the product, this is dissolved in 100 volumes (90 l) acid pH solvent mixture, consisting of: 90 volumes (81 l) ethyl alcohol 95^C + 10 volumes (9 l) "I" structured water (pH=1.8...2.6), by heating at 80^0C, for 1 h, the resulted yellow solution is treated with 0.1...0.3% m/v acid active carbon, filtered, vacuum concentrated, at 35...40^0C, up to 1/10 of the initial volume (9 l), when a microcrystalline suspension is resulted, which is kept for 24 h at 5...10^0C for finishing crystallization, afterwards it is vacuum filtered, the precipitate is washed with 0.4l distilled water, up to neutral pH, dried at 105^0C for 3h and then pulverized.

Minimum 560 g white, microcrystalline powder are produced, consisting of crystallized bioactive complex of triterpene acids, with a minimum 85% content, purified by recrystallization in the following way: the substance is dissolved in 100 volumes (56 l) solvent mixture consisting of: 90 volumes (50.4 l) ethyl alcohol 95^C + 10 volumes (5.6 l) "I" structured water (pH=1.8...2.6) by heating at 80^0C, for 1 h and the solution obtained is vacuum concentrated, at 35...40^0C, up to 1/5 of the initial volume (11.2 l), resulting in a microcrystalline suspension, which is kept for 24 h at 5...10^0C for finishing crystallization, then is vacuum filtered, the filter precipitate is washed with 0.4 l distilled water, up to neutral pH, dried at 105^0C for 3 h and then pulverized. Minimum 490 g white, microcrystalline powder are produced, constituted of crystallized bioactive complex of triterpene acids, with a 93.7% content formed of: 77.5% ursolic acid, 10.1% oleanolic acid and 6.1% other triterpene acids, consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 50% final efficiency, compared with 3% content of triterpene acids in the processed plant raw material. This bioproduct is used for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Variant B

30 kg dried and pulverized plant raw material, consisting of *herba Lavandula species* (*Lavandula angustifolia, Lavandula vera, Lavandula spica, Lavandula fragrans, Lavandula latifolia,* etc) or of lavender residue resulted from production of essential oil by steaming, with a 2...4% content of triterpene acids, are extracted with 500 l extraction solvent, consisting of: 90 volumes (450 l) ethyl alcohol 95^C + 10 volumes (50 l) "I" structured water (pH=1.8...2.6) by dynamic maceration, for 12 h, at ambient temperature (15...30^0C), then minimum 400 l extractive solution are collected which are passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated, at 35...40^0C, up to the

40 l volume, a suspension being obtained which is kept for 24 h at 5...10^{0}C, then vacuum filtered, and the filter precipitate is dried at 105^{0}C for 3 h and pulverized. Minimum 900 g greenish powder are obtained which constitute the crude bioactive complex of triterpene acids with a minimum 70% content.

To purify the product, it is dissolved in 100 volumes (90 l) acid pH solvent mixture, composed of : 90 volumes (81 l) acetone + 10 volumes (9 l) "I" structured water with pH=1.8...2.6, the suspension is heated at 80^{0}C for 1 h until full substance dissolution, and the resulted yellow solution is treated with 0.1...0.3% m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated, at 35...40^{0}C, up to 1/10 of the initial volume (9 l), when a microcrystalline suspension is obtained, which is kept for 24 h at 5...10^{0}C for finishing crystallization, then it is vacuum filtered, the precipitate is washed with 0.4 l distilled water, up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 570 g white, microcrystalline powder are obtained, consisting of bioactive complex of triterpene acids, with a minimum 85% content, which is purified by recrystallization in the following way: the substance is dissolved in 100 volumes (57 l) solvent mixture consisting of : 90 volumes (51.3 l) ethyl alcohol 95^C + 10 volumes (5.7 l) "I" structured water (pH=1.8...2.6), by heating at 80^{0}C for 1 h and the obtained solution is vacuum concentrated at 35...40^{0}C, up to 1/5 of the initial volume (11.4 l), resulting in a microcrystalline suspension which is kept for 24 h at 5...10^{0}C for finishing crystallization, afterwards it is vacuum filtered, the filter precipitate is washed with 0.4 l distilled water, up to neutral pH, dried at 105^{0}C for 3 h and then pulverized.

Minimum 480 g white, microcrystalline powder are obtained, consisting of crystallized bioactive complex of triterpene acids, with a 94.2% content formed of: 78.1% ursolic acid, 11.2% oleanolic acid and 4.9% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 50% final efficiency compared with the 3% content of triterpene acids of processed plant raw material.

This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.3 – Variant A

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS AT INDUSTRIAL SCALE FROM FLOWERS OR LEAVES OF *SAMBUCUS NIGRA* (*ELDER TREE*)

30 kg dried and pulverized plant raw material consisting of flowers or leaves of *Sambucus nigra*, with a content of 2...3% triterpene acids, are extracted with 500 l extraction solvent, composed of: 90 volumes (450 l) acetone + 10 volumes (50 l) "I" structured water with pH=1.8...2.6 by dynamic maceration, for 12 h, at ambient temperature (15...30^{0}C), then minimum 400 l extractive solution are collected and then passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated at 35...40^{0}C up to the 40 l volume, a suspension being obtained which is kept for 24 h at 5...10^{0}C, then vacuum filtered and the filter precipitate is dried at 105^{0}C for 3 h and then pulverized.

Minimum 600 g greenish powder are obtained, constituting the crude bioactive complex of triterpene acids with a minimum 70% content.

For purification of the product, the solvent mixture is used with acid pH, consisting of 90 volumes (54 l) ethyl alcohol 95^C + 10 volumes (6 l) "I" structured water with pH=1.8...2.6 in which 600 g powder are added for dissolution, consisting of crude bioactive complex of triterpene acids (100 ml solvent mixture being calculated for 1 g substance); suspension is heated at 80^{0}C, for 1 h, until full substance dissolution, and the resulted yellow solution is treated with 0.1...0.3% m/v acid active carbon; the suspension is filtered, the obtained solution is vacuum concentrated, at 35...40^{0}C, up to 1/10 of the initial volume (6 l), when a microcrystalline suspension is achieved which is kept for 24h at 5...10^{0}C, for finishing crystallization, then it is vacuum filtered, the precipitate is washed with 0.3 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 400 g white, microcrystalline powder are obtained, consisting of the bioactive complex of triterpene acids, with a minimum 85% content, which is purified by recrystallization in the following way: substance is dissolved in 100 volumes (40 l) solvent mixture made of 90 volumes (36 l) ethyl alcohol 95^C + 10 volumes (4 l) "I" structured water (pH=1.8...2.6) by heating at 80^{0}C for 1h and the obtained solution is vacuum concentrated, at 35...40^{0}C, up to 1/5 of the initial volume (8 l), resulting in a microcrystalline suspension, which is vacuum filtered, the filter precipitate is washed with 0.3 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 330 g white, microcrystalline powder are obtained, consisting of: crystallized bioactive complex of triterpene acids, with a 91.8% content formed of: 76.2% ursolic acid, 11.1% oleanolic acid and 4.5% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 50% final efficiency, compared with the

2% triterpene acids content of processed plant raw material. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Variant B

30 kg dried and pulverized plant raw material, consisting of flowers or leaves of *Sambucus nigra*, with a 2...3% content of triterpene acids, are extracted with 500 l extraction solvent, made of: 90 volumes (450 l) ethyl alcohol 95^C + 10 volumes (50 l) "I" structured water (pH=1.8...2.6), by dynamic maceration, for 12 h, at ambient temperature (15...30^0C), then minimum 400 l extractive solution are collected, passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated, at 35...40^0C, up to the 40 l volume, a suspension being obtained which is kept for 24 h at 5...10^0C, then is vacuum filtered and the filter precipitate is dried at 105^0C for 3 h and pulverized. Minimum 600 g slightly greenish-yellow powder are produced, constituting the crude bioactive complex of triterpene acids, with a minimum 70% content.

For purification of the crude product, the acid pH solvent mixture is used, consisting of 90 volumes (54 l) acetone + 10 volumes (6 l) "I" structured water with pH=1.8...2.6 in which 600 g powder are added for dissolution, consisting of crude bioactive complex of triterpene acids (100 ml solvent mixture are calculated for 1 g substance); suspension is heated at 80^0C for 1 h until full substance dissolution, and the resulted yellow solution is treated with 0.1...0.3% m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated, at 35...40^0C, up to 1/10 of the initial volume (6 l), when a microcrystalline suspension is produced which is kept for 24 h at 5...10^0C for finishing crystallization, afterwards it is vacuum filtered, the precipitate is washed with 0.3 l distilled water up to neutral pH, dried at 105^0C for 3 h and pulverized.

Minimum 400 g white, microcrystalline powder are obtained, consisting of bioactive complex of triterpene acids, with a minimum 85% content, which is purified by recrystallization in the following way: substance is dissolved in 100 volumes (40 l) solvent mixture made of: 90 volumes (36 l) ethyl alcohol 95^C + 10 volumes (4 l) "I" structured water (pH=1.8...2.6), by heating at 80^0C, for 1 h, and the obtained solution is vacuum concentrated, at 35...40^0C, up to 1/5 of the initial volume (8 l), resulting in a microcrystalline suspension, which is vacuum filtered, the filter precipitate is washed with 0.3 l distilled water, up to neutral pH, dried at 105^0 C for 3 h and pulverized.

Minimum 325 g white, microcrystalline powder are produced, consisting of crystallized bioactive complex of triterpene acids, with a 91.5% content formed of: 77.1% ursolic acid, 10.3% oleanolic acid and 4.1% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 50% final efficiency as compared with the 2% triterpene acids content of processed plant raw material. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.4 – Variant A

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS FROM *HERBA CRATAEGUS SPECIES*

30 kg dried and pulverized plant raw material consisting of herba (leaves and flowers) of *Crataegus sp.* with a 2.5...3.5% content of triterpene acids, are extracted with 500 l extraction solvent, composed of: 90 volumes (450 l) acetone + 10 volumes (50 l) "I" structured water with pH=1.8...2.6 by dynamic maceration, for 12 h, at ambient temperature (15...30^{0}C), afterwards minimum 400 l extractive solution are collected which are passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated at 35...40^{0}C, up to the 40 l volume, a suspension being obtained which is kept for 24 h at 5...10^{0}C, then is vacuum filtered and the filter precipitate is dried at 105^0 for 3 h and pulverized.

Minimum 1000 g greenish powder are produced which constitute the crude bioactive complex of triterpene acids, with a minimum 70% content.

For purification of the product, the acid pH solvent mixture is used, consisting of 90 volumes (90 l) ethyl alcohol 95^C + 10 volumes (10 l) "I" structured water with pH=1.8...2.6, in which 1000 g powder is added for dissolution, composed of crude bioactive complex of triterpene acids (100 ml solvent mixture are calculated for 1 g substance); suspension is heated at 80^{0}C for 1 h until full substance dissolution, and the resulted yellow substance is treated with 0.1...0.3% m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated at 35...40^{0}C up to 1/10 of the initial volume (10 l), when a microcrystalline suspension is produced which is kept for 24 at 5...10^{0}C, for finishing crystallization, then vacuum filtered, the precipitate is washed with 0.2 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 500 g white, microcrystalline powder are obtained, composed of bioactive complex of triterpene acids with a minimum 85% content which is purified by recrystallization in the following way: substance is dissolved in 100 volumes (50 l) solvent mixture consisting of 90 volumes (45 l) ethyl alcohol 95^C + 10 volumes (5 l) "I" structured water (pH=1.8...2.6), by heating at 80^0C for 1 h, and the obtained solution is vacuum concentrated at 35...40^0C up to 1/5 of the initial volume (10 l), resulting in a microcrystalline suspension which is vacuum filtered, the filter precipitate is washed with 0.2 l distilled water up to neutral pH, dried at 105^0C, for 3 h, and pulverized.

Minimum 450 g white, microcrystalline powder are produced, composed of crystallized bioactive complex of triterpene acids with a 92.6% content, consisting of: 76.5% ursolic acid, 12% oleanolic acid and 4.1% other triterpene acids formed of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 55% final efficiency, as compared with the 2.5% triterpene acids content of processed plant raw material. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Variant B

30 kg dried and pulverized plant raw material, consisting of herba (leaves and flowers) of *Crataegus sp.* with a 2.5...3.5% content of triterpene acids, are extracted with 500 l extraction solvent, consisting of: 90 volumes (450 l) ethyl alcohol 95^C + 10 volumes (50 l) "I" structured water (pH=1.8...2.6), by dynamic maceration for 12 h, at ambient temperature (15...30^0C), then minimum 400 l extractive solution are collected, which are passed on a chromatographic column with 2 kg acid active granulated carbon, resulting in a purified extractive solution, which is vacuum concentrated, at 35...40^0C, up to the 40 l volume, a suspension being obtained which is kept for 24 h at 5...10^0C, then it is vacuum filtered and the filter precipitate is dried at 105^0C for 3 h and pulverized. Minimum 1000 g slightly greenish-yellow powder which constitute the crude bioactive complex of triterpene acids with a minimum 70% content.

For purification of the crude product, the acid pH solvent mixture is used, consisting of 90 volumes (90 l) acetone + 10 volumes (10 l) "I" structured water with pH=1.8...2.6 where 1000 g powder, composed of crude bioactive complex of triterpene acids (100 ml solvent mixture are calculated for 1 g substance), are added for dissolution; suspension is heated at 80^0C for 1h until full substance dissolution and the

resulted yellow solution is treated with 0.1...0.3 m/v acid active carbon; suspension is filtered, the obtained solution is vacuum concentrated at 35...40^{0}C up to 1/10 of the initial volume (10 l) when a microcrystalline suspension is produced which is kept for 24 h at 5...10^{0}C, for finishing crystallization, then it is vacuum filtered, the precipitate is washed with 0.2 l distilled water, up to neutral pH, dried at 105^{0}C for 3 h and pulverized. Minimum 500 g white, microcrystalline powder, are obtained, constituted from bioactive complex of triterpene acids with a minimum 85% content, which is purified by recrystallization as follows: the substance is dissolved in 100 volumes (50 l) solvent mixture consisting of: 90 volumes (45 l) ethyl alcohol 95^C + 10 volumes (5) "I" structured water (pH=1.8...2.6) by heating at 80^{0}C for 1 h and the obtained solution is vacuum concentrated at 35...40^{0}C up to 1/5 of initial volume (10 l), resulting in a microcrystalline suspension which is vacuum filtered, the filter precipitate is washed with 0.2 l distilled water, up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 430 g white, microcrystalline powder are obtained, consisting of crystallized bioactive complex of triterpene acids, with a 93% content formed of: 77.8% ursolic acid, 10.7% oleanolic acid and 4.5% other triterpene acids consisting of one or more of the following acids: hydroxyrusolic, hydroxyoleanolic, betulinic, dehydrobetulinic, which represents a minimum 53% final efficiency as compared with the 2.5% triterpene acids content of processed plant raw material. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.5

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS IN THE FORM OF Na SALT

200 g powder of crystallized bioactive complex of triterpene acids, with a minimum 90% content, produced according to the working process described in the examples nos.1,2 and 3 are dissolved in 20 l (1/100 m/v ratio) solvent mixture consisting of: 90 volumes (18 l) ethyl alcohol 95^C + 10 volumes (2 l) "S" structured water (pH=10...12), containing 1% NaOH, by reflux heating (80^{0}C), a clear solution being obtained which is vacuum concentrated, at 40...45^{0}C, up to 1/4 of the initial volume (5 l), a microcrystalline solution being produced which is kept for 24 h at 5...10^{0}C for finishing crystallization and then it is vacuum filtered, the filter precipitate is washed with minimum 0.1 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized.

Minimum 190 g white, microcrystalline powder are obtained, consisting of crystallized bioactive complex of triterpene acids, in the form of Na salt of ursolic, oleanolic acids and other triterpene acids formed of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, with a 90% content, which represents a 95% phase efficiency. This bioproduct is usable for conditioning some medicinal drugs for internal use (tablets, capsules, injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.6

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS IN THE FORM OF K SALT

200 g crystallized bioactive complex of triterpene acids, with a minimum 90% content, produced according to the working process described in the examples nos.1,2 and 3 are dissolved in 20 l solvent mixture consisting of: 90 volumes (18 l) ethyl alcohol 95^C + 10 volumes (2 l) "S" structured water (pH=10...12), containing 1% KOH, by reflux heating (80^0C); the resulted clear solution is vacuum concentrated at 40...45^0C up to 1/4 of the initial volume (5 l), a microcrystalline suspension being produced which is kept for 24 h at 5...10^0C, for finalizing crystallization, then it is vacuum filtered, the filter precipitate is washed with minimum 0.1 l distilled water up to neutral pH, dried at 105^0C for 3 h and pulverized. Minimum 190 g white, microcrystalline powder are obtained, consisting of bioactive complex of triterpene acids, in the form of K salt of ursolic, oleanolic acids and other triterpene acids composed of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic with a 90% content, which represents a 95% phase efficiency.

The bioproduct is usable for conditioning some medicinal drugs for internal use (injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

Example no.7

PRODUCTION OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS IN THE FORM OF NH_4 SALT

200 g crystallized bioactive complex of triterpene acids, with a minimum 95% content, produced according to the working process described in the examples nos.1, 2 and 3, are dissolved in 20 l solvent mixture consisting of : 90 volumes (18 l) ethyl alcohol 95^C + 10 volumes (2 l) "S" structured water (pH=10...12), containing 5% NH_4OH 25%, by reflux heating (80^0C); the resulted clear solution is vacuum concentrated at 40...45^0C up to 1/4 of the initial volume (5 l), a microcrystalline suspension being

produced which is kept for 24 h, at 5...10^{0}C for finishing crystallization, then it is vacuum filtered, the filter precipitate is washed with minimum 0.1 l distilled water up to neutral pH, dried at 105^{0}C for 3 h and pulverized. Minimum 190 g white, microcrystalline powder are produced, composed of bioactive complex of triterpene acids, in the form of NH_4 salt of ursolic, oleanolic acids and other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, with a minimum 90% content, which represents a 95% phase efficiency. The bioproduct is usable for conditioning some medicinal drugs for internal use (injectable and buvable solutions) or for external use (eye washes, ophthalmic gel, spray).

TESTS FOR ANALYTICAL QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF THE BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Qualitative and quantitative characterization of the bioactive complex of triterpene acids produced according to the invention, was made by application of the following analytical methods :

- Thin layer chromatography (TLC);
- VIS molecular absorption spectrometry;
- High Performance Liquid Chromatography (HPLC);
- Gas-chromatography coupled with mass spectrometry (GC-MS);
- Nuclear magnetic resonance ^{13}C (^{13}C-NMR);

Test no. 1 - Qualitative evaluation of bioactive complex of triterpene acids by TLC

Reagents:

- Silicagel 60 F_{254} (Merk) chromatographic plates;
- Mobile phase: cyclohexane-acetone-ethyl acetate (4:2:1) v/v;
- Identification reagent : chloroform-acetic anhydride-sulphuric acid (50 :10 :1) v/v ;
- Reference solution: 0.1 g ursolic acid – reference substance in 100 ml methyl alcohol;
- Test solution: 0.1 g bioactive complex of triterpene acids – test sample in 100 ml methyl alcohol.

Method:

Every 50 ml reference solution and 50 ml test solution are applied on chromatographic plate and then they are put in chromatographic vessel containing the mobile phase, allowing the solvent front to migrate a minimum 15 cm distance and after drying at 105^{0}C, being sprayed with identification reagent.

Experimental results:

On the chromatographic plate, 2 red-violaceous primary spots with R_f=0.75, are seen, corresponding to the reference substance and test sample, that also show 2...4 less intense, reduced surface, violaceous coloured secondary spots, with R_f=0.74; 0.72; 0.71 and 0.64, belonging to other free-state, non-glycoside triterpene acids (i.e. oleanolic, hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic), accompanying ursolic acid in the extraction, purification and crystallization processes.

Test no. 2 - Quantitative determination of bioactive complex of triterpene acids by VIS molecular absorption spectrometry

Reagents:

- 5% vanillin solution in acetic acid;
- Perchloric acid (R);
- Reference solution: 0.1 g ursolic acid – reference substance in 100 ml methyl alcohol;
- Test solution: 0.1 g bioactive complex of triterpene acids-test sample in 100 ml methyl alcohol.

Method:

0.1 g bioactive complex of triterpene acids – test sample is dissolved in 100 ml methyl alcohol. Out of the solution obtained, 5 ml are taken that are dissolved in 50 ml graduated flask with methyl alcohol (solution A).

5 ml of solution A are diluted in 50 ml graduated flask with methyl alcohol (solution B).

1 ml of solution B is evaporated on water bath at a temperature of 70^0C up to sicc residue, over which there are added: 0.2 ml of 5% vanillin solution in acetic acid and 1 ml perchloric acid and they are heated on water bath at 60^0C for 15 minutes; after cooling at room temperature, acetic acid is added up to 5 ml, then homogenized and absorbance is read at 550 nm as against a control solution prepared in the same way but containing 1 ml methyl alcohol instead of sample.

Bioactive complex of triterpene acids content is determined by means of a standard curve of ursolic acid – reference substance, as 0.1% solution in methyl alcohol, the following calculating formula being applied:

$$\text{Bioactive complex of triterpene acids content \%} = \frac{Ap \cdot Csr}{Asr \cdot P} x\,10^{-2}$$

expressed in ursolic acid

where:

Ap = absorbance of Bioactive complex of triterpene acids solution – sample at 550 nm;

Asr = reference solution absorbance at 550 nm;

Csr = reference substance concentration – µg/ml corresponding to Asr absorbance;

P = test sample quantity – g

10^{-2} = corrective factor

Experimental results

Quantitative determination of Bioactive complex of triterpene acids by VIS molecular absorption spectrometry led to the following experimental results shown in Table no. 1

Table no. 1- Total content of bioactive complex of triterpene acids % - expressed in ursolic acid

Processed plant	*Salvia species*		*Lavandula species*		*Sambucus nigra*		*Crataegus species*	
Sample of bioactive complex of triterpene acids no	1A	1B	2A	2B	3A	3B	4A	4B
Content of bioactive complex of triterpene acids %	96.6	96.7	93.7	94.2	91.8	91.5	92.6	93.0

From the above table, one can see that the highest total content of bioactive complex of triterpene acids was found in samples of Salvia species, followed by *Lanvandula, Crataegus and Sambucus nigra species.*

Test no. 3 - Quantitative determination of bioactive complex of triterpene acids, by HPLC

Method:

By HPLC, quantitative determination of bioactive complex of triterpene acids was made, applying external standard method, using an Agilent 1100 chromatographic system provided with diode series detector and fraction collector.

The samples of bioactive complex of triterpene acids were subject to separation on Hypersil C_{18} reversed phase chromatographic column and phase mobile elution, consisting of the mixture of methanol / formic acid 0.1 M (90/10 v/v); UV detection was recorded at 205 nm.

Experimental results:

Experimental data obtained are shown in Table no. 2, where one can see that the highest content of ursoslic and oleanolic acids (91.1... 92.1%) was found in samples

of bioactive complex of triterpene acids taken from *Salvia species*, followed by the other tested samples, with values of 87.3 – 89.3%.

Applying the difference between results of spectrophotometric analysis and of HPLC one, there was indirectly determined a 4...10% content of other triterpene acids, present beside ursolic and oleanolic acids, in samples of bioactive complex of triterpene acids produced according to the invention.

Table no. 2 - Quantitative determination of bioactive complex of triterpene acids, by HPLC

Bioactive complex of triterpene acids						
Salvia species	Sample 1 A			Sample 1 B		
	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids* (%)	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids * (%)
	80.9	10.2	5.5	80.4	11.7	4.6
Lavandula species	Sample 2 A			Sample 2 B		
	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids* (%)	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids * (%)
	77.5	10.1	6.1	78.1	11.2	4.9
Sambucus nigra	Sample 3 A			Sample 3 B		
	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids* (%)	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids * (%)
	76.2	11.1	4.5	77.1	10.3	4.1
Crataegus species	Sample 4 A			Sample 4 B		
	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids* (%)	Ursolic acid (%)	Oleanolic acid (%)	Other triterpene acids * (%)
	76.5	12	4.1	77.8	10.7	4.5

*Quantitatively evaluated indirectly on the basis of the difference of results obtained by spectrophotometric method and HPLC.

By half-preparative liquid chromatographic method, GC-MS and ^{13}C-MNR, it was qualitatively determined that, the 4...10% total content of other triterpene acids present in bioactive complex of triterpene acids, beside ursolic and oleanolic acids, consists of 2...4 of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic, as resulted from chromatograms shown in Figure 1.

ANALYTICAL CHARACTERISTICS OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Crt. No.	Analytical characteristics	Quality standard specifications
1.	Definition	Complex of triterpene acids: ursolic, oleanolic and others with a similar structure
2.	Physical properties	
	- description	White, tasteless and odourless, non-hygroscopic microcrystalline powder
	- solubility	Water insoluble powder, sparingly soluble in alcohols at ambient temperature, soluble at reflux temperature in alcohols, acetone and non-polar organic solvents
3.	Identification	
	- by thin-layer chromatography	
	- ursolic acid	Primary spot with R_f=0,75
	- oleanolic acid	Secondary spot with R_f=0,74
	- other triterpene acids	2...3 less intense secondary spots, with R_f = 0,72; 0,71; 0,64
4.	Quantitative determination	
	A. Spectophotopmetric method	
	- Total content of triterpene acids - %	90...98
	B. HPLC Method	
	- content of ursolic and oleanolic acid - %	75...80 ursolic acid 10...15 oleanolic acid
5.	Loss on drying	Max. 1%
6.	Calcination residue	Max. 0,1%

7.	Purity control	
	- heavy metals	Max. 10 ppm
	- organic solvent residue	Absent
8.	Microbial contamination	
	- no. of total aerobic bacteria, ufc/ml	Max. 10^4
	- no. of total yeasts and filamentous fungi /ml	Max. 10^2
	- potential pathogenic bacteria	
	• Enterobacteriaceae/ml	Max. 10^2
	• Staphylococcus aureus/ml	Absent
	• Escherichia coli/ml	Absent
	• Salmonella/10 g	Absent
9.	Preservation	Min. 3 years, at ambient temperature (20-25^{0}C), in air tight containers, protected from light

PRECLINICAL TOXICOLOGICAL AND PHARMACOLOGICAL TESTS

"In vivo" preclinical pharmacotoxicological tests aimed at evaluating acute toxicity of bioactive complex of triterpene acids, on two species of rodents: mouse and rat, and at revealing the bioproduct specific activity in young and old Wistar rats and Chinchilla rabbits.

Pharmacotoxicological studies revealed that the toxic potential (pT) value of bioactive complex of triterpene acids is lower than 2.00 (pT<1.85), which makes the bioproduct to be included in the group of "actually non-toxic" substances.

Further on, the result of tests on specific pharmacological activity of bioactive complex of triterpene acids, is shown.

A. METABOLIC BIOREGULATING ACTIVITY ON AGEING PROCESSES

Considering the fact that a number of medicinal herbs belonging to *Labiatae* family, containing triterpene acids as prevalent bioactive components, are used in traditional medicine of various countries and as remedies for ageing prevention or treatment, pharmacological studies on specific activity of bioactive complex of triterpene acids (CBAT) were directed to evidencing and evaluating the influence of bioactive complex of triterpene acids (CBAT) on prevention and/or slowing down of ageing processes.

I. BIOREGULATING ACTIVITY ON PROTEINIC METABOLISM

Although biological and biochemical phenomena related to ageing process have not been cleared up yet, results of experimental studies reveal importance of some factors responsible for senescence occurrence, among which the following ones can be mentioned:

- protein synthesis with an altered structure, due to functional deficiency of enzymes catalyzing various stages of proteinic synthesis;

- effects of some analogues of amino acids and nitrogenous bases, components of nucleic acids, on longevity; thus, it was demonstrated by Doug, Xoaping et. al (Yaown Feuxi Zazhi 1985, 5(1) 45-46, CH) that analogues of amino acids (i.e. DL – ethionine, DL-seleno-methionine) can be included in polypeptide chain of enzymatic proteins, diminishing their activity and causing significant shortening of their lifetime .

Tests applied with a view to evidencing the influence of bioactive complex of triterpene acids (CBAT) on proteinic metabolism, consisted in evaluating collagen serum proteins in young and old Wistar rats, under normal breeding conditions and also under conditions of stimulating ageing processes through a mechanism for diminishing enzymatic proteins activity by administering DL-ethionine. Also, the dose-effect relationship was followed at the level of content and synthesis of liver proteins in old Wistar rats. Experimental data obtained are shown in tables 1, 2.

The following are concluded from the table:

- DL-ethionine administration influences differently animals weight gain, in terms of their age, the old animals showing slowing down in growing rate by 12.75% against control group, as compared with 5.09% in young animals;

Table no.1 – Effect of treatment with bioactive complex of triterpene acids (CBAT) on blood and hide proteins in young and old Wistar rats, under conditions of stimulating ageing processes with DL-ethionine.

Significance of media difference by Student "t"- test at $p<0.05$

Age of Wistar rats	Group	Parameter				
		Initial body weight (g)	Final body weight, (g)	Weight variation against control (%)	Total proteins, (g %)	Hide soluble collagen (mg/100g hide)

Young rats	Control group (n=10)	119.0 ± 3.20	130.8 ± 2.25	-	7.22 ± 0.32	968±83
	DL-ethionine 10 mg/kg b.w. (n = 10)	122.4 ±3.85	133.6 ±3.46	- 5.09	6.15 ±0.48	941±101
	DL-ethionine 10 mg/kg b.w.+CBAT 50mg/kg b.w. (n=10)	120.8 ±1.98	135.9 ±2.76	- 1.49	7.95 ±0.64	974±120
Old rats	Control group (n=10)	182.3 ±2.23	192.5 ±1.86	-	6.72 ±0.31	780±68
	DL-ethionine 10mg/kg b.w. (n = 10)	179.2 ±1.84	188.1 ±2.36	- 12.75	5.16 ±0.26[a]	614±114
	DL-ethionine 10mg/kg b.w. +CBAT 50 mg/kg b.w. (n = 10)	180.8 ±3.62	194.5 ±1.64	- 0.83	8.08 ±0.40[b]	818±154

[a]$p < 0.05$; [b]$p < 0.005$ vs. control group

- treatment with bioactive complex of triterpene acids (CBAT) administered simultaneously with DL-ethionine determines a more intense increase of growing rate in old animals as against the young ones;

- CABT selective efficiency on old animals is also demonstrated when evaluating the total serum proteins, as well as hide soluble collagen fraction in the animals which were administrated DL-ethionine; data obtained show significant decrease of hide serum proteins and soluble collagen in the case of old rats in which CBAT treatment stimulates proteinic synthesis.

TABLE no.2 – Effect of the treatment with bioactive complex of triterpene acids (CBAT) on liver proteins in old Wistar rats

Significance of media difference by Student "t"- test at $p<0.05$

Group	Parameter			
	Initial liver proteins, (mg/g tissue)	Difference (%)	Liver proteins synthesis (dpm/mg prot.)	Diffe-rence (%)
Control group, (n=10)	155.4 ± 3.18	-	5.76 ± 0.69	-
CBAT 50 mg/kg b.w. (n=10)	168.8 ± 4.96[a]	+8.62	8.11 ± 0.78[b]	+ 40.8
CBAT 100 mg/kg b.w (n=10)	182.2 ± 7.2[a]	+17.24	8.78 ± 0.91[b]	+ 52.4

[a]p = 0.01, [b]p=0.001 vs. control group

From the table one can conclude that administration of bioactive complex of triterpene acids determines increase of liver proteins content, in terms of dose, correlated with enhancing liver protein synthesis in old animals in which this process is slowed down.

The results achieved and shown in tables nos.1 and 2, demonstrate that the bioactive complex of triterpene acids acts selectively on old organisms, restoring changes caused by age in proteinic metabolism, which motivates curative therapeutic use of this bioproduct in degenerative processes of vascular, articular, tissular and dermic collagen and in geriatric preventive therapy as well.

II. BIOREGULATING ACTIVITY ON GLUCIDIC METABOLISM

Study of the influence of bioactive complex of triterpene acids on glucidic metabolism was performed by testing its hypoglycemic effects on experimental diabetes mellitus in white Wistar rats, induced by means of two experimental models: with streptozotocin and with prednisone, respectively.

a. Diabetes mellitus induced by streptozotocin

Induction of streptozotocin diabetes was carried out by classical method used in diabetes research, that provides intravenous administration in white Wistar rats deprived of feed for 18 hours, of a streptozotocin solution in 0.01 M citrate buffer, in a dose of 50 mg/100 g b.w.; after 1 h, 1 ml 30% glucose aqueous solution was intraperitoneally given, and in 24 h after injecting streptozotocin, the experimentally induced diabetes was examined.

The group of experimentally induced diabetes animals was treated with bioactive complex of triterpene acids in a dose of 50 mg/100 g b.w., for 10 days, and then the glucidic metabolism parameters were investigated: glycemia, liver glycogen and histologic aspects of pancreas, thyroid, spleen and thymus, as main endocrine glands

involved in glucidic homeostasis. To evaluate antioxidating activity, the liver superoxide – dismutase (SOD) level was determined.

Experimental data obtained are shown in table no.3

TABLE no.3. Effect of treatment with bioactive complex of triterpene acids (CBAT) on some parameters of glucidic metabolism in white Wistar rats with experimentally streptozotocin - induced diabetes.

Significance of media difference by Student "t" – test at $p < 0.05$

Group	Parameter				
	Glycemia (mg %)	Difference against control group (%)	Liver glycogen (mg/100 g tissue)	Difference against control group (%)	Liver SOD U/mg proteins
LM (n=12)	84.22 ± 4.08	-	567 ± 20.82	-	45.2 ± 1.3
LS (n=12)	190.08±4.93[a]	+125.69	1596± 80.33[a]	+181.48	36.9± 2.1[a]
LSCBAT (n=12)	170.16±7.28[a,b]	+102,04	1218±29.54[a,c]	+114.81	43.6± 1.9[b]

LM =normal control group;

LS = control group with streptozotocin - induced diabetes 50 mg/100 g.b.w.

LSCBAT = group with CBAT treated diabetes 50 mg/100g b.w./day, 10 days

[a]$p < 0.001$ vs normal control group;

[b]$p < 0.05$ and [c]$p < 0.001$ vs control group with diabetes

Data obtained and shown in table no.3. reveal hypoglycemic effect of bioactive complex of triterpene acids, exhibited by both a decrease in circulating glucose quantity and especially by a significant diminution of liver glycogen in the group of Wistar rats with induced diabetes, treated with bioactive complex of triterpene acids (CBAT). Results of histological investigations on endocrine pancreas, thyroid, spleen and thymus showed that under streptozotocin-diabetes conditions, administration of bioactive complex of triterpene acids determines a protective action on morphofunctionality of these organs, materialized by counteracting toxic effects at streptozotocin cellular level and preventing alterations at nucleic acids level.

b. Steroidal diabetes mellitus induced by prednisone

On the basis of experimental studies showing that endogenous or exogenous excess of glucocorticosteroids causes diabetogenous and antiinsulin effects, exhibited

by hyperglycemia, high liver gluconeogenesis and tissue insulin resistance, the hypoglycemic activity of bioactive complex of triterpene acids was investigated by applying an experimental model for inducing diabetes mellitus in white Wistar rats, by intragastric administration of prednisone-acetate, in doses of 3mg/100 g b.w./day, for 10 days, as compared with a group that was induced diabetes under the same conditions, then treated with bioactive complex of triterpene acids, in doses of 50 mg/100 g. b.w./day, for 10 days, and afterwards biochemical determinations of glycemia and total serum cholesterol, were effected considering the glucocorticosteroids hypercholesterolemic effect.

Experimental results achieved are shown in table no.4.

From table no.4 one can conclude that in rat group with experimentally prednisone-induced diabetes, simultaneous administration of bioactive complex of triterpene acids results in a significant decrease of glycemia and a return to normal values of total serum cholesterol, the tested bioactive product annihilating prednisone hypercholesterolemic effect. These results, corroborated with those shown in table no.3, demonstrate that bioactive complex of triterpene acids influences beneficially glucidic metabolism, having hypoglycemic effects on experimental diabetes and hypocholesterolemic ones as well, which motivates bioproduct application in antidiabetes therapy, associated with hyperlipidemia, especially in diabetic obesity.

TABLE no.4. – Effect of treatment with bioactive complex of triterpene acids (CBAT) on glycemia and total serum cholesterol in white Wistar rats with prednisone - induced experimental diabetes.

Significance of media difference by Student "t" – test at $p < 0.05$

Group	Parameter			
	Glycemia (mg%)	Difference as against control group (%)	Serum cholesterol (mg %)	Difference as against control group (%)
LM (n=10)	75 ± 3.86	-	119 ± 3.02	-
LP (n=10)	140 ± 3.18[a]	+ 86.66	160 ± 2.30[a]	+34.45
LPCBAT (n=10)	105 ± 4.42[a,b]	+40.00	120 ± 2.85[a,b]	+0.84

LM = normal control group; LP= control group with diabetes induced by prednisone with 3mg/100 g b.w/day, 10 days; LPCBAT= group with CBAT-treated diabetes with 50 mg/100 g b.w./day, 10 days.

[a]$p<0.001$ vs normal control group; [b]$p< 0.001$ vs diabetes control group

III. BIOREGULATING ACTIVITY ON LIPIDIC METABOLISM

Hypolipidemic and antiatheromatous action of bioactive complex of triterpene acids was evident on an experimental model of atheromatosis in rabbit, where some of risk factors of human pathology were associated in atherogenic diet. Experiment was carried out on Chinchilla breed adult rabits, distributed in the following groups:

LMr = control group – standard diet, 42 days;

LNA = control group with atheromatosis – atherogenic diet, 42 days;

LF = group with simultaneous administration of atherogenic diet + Fluvastatin 1.5 mg/kg b.w. per oral/day;

LCBAT = group with simultaneous administration of atherogenic diet + 50 mg CBAT/kg b.w.per oral/day.

Within the experiment, the influence of bioactive complex of triterpene acids was investigated compared with that one of Fluvastatin, on hypolipidemia induced by administration of atherogenic diet, through evaluation of atheromatosis extent at aorta level and measurement of the following blood biochemical parameters: triglycerides, total cholesterol, lipoproteins LDL and HDL, cholesterol/HDL ratio, transaminases (TGO, TGP), creatine phosphokinase, the following results being obtained:

TABLE no.5. – Effect of bioactive complex of triterpene acids compared with Fluvastatin on atheromatosis process in rabbits fed with atherogenic diet

No. animal/group and animal sex	Estimating score of atheromatosis extent at aorta level			
	LMr	LMA	LF	LCBAT
1M	1	2	0	0
2M	1	4	1	3
3M	0	4	2	2
4F	1	2	1	0
5F	0	1	1	3
6F	0	3	0	0
Score/group	3	16	5	8

0 points = without lesions of endarterium

1 point = one or more small plates (below 1 mm)

2 points = 1 big plate (over 1 mm) and more small plates

3 points = more big plates;

4 points = most of aorta injured

TABLE no. 6 – Hypolipidemic action of bioactive complex of triterpene acids (CBAT) as against Fluvastatin, on some lipidic fractions in rabbits fed with atherogenic diet

Significance of media difference throught Student „t" – test at $p < 0.05$

Group	Parameters			
	Cholesterol (mg/dl)	TG (mg/dl)	Lipo LDL (mg/dl)	Cholesterol/HDL
LMr (n=6)	95.33 ± 4.72	145.00 ± 38.94	48.62 ± 16.67	7.09 ± 2.13
LMA (n=6)	948.50± 180.75[a]	222.83± 62.97[b]	877.55± 180.51[a]	29.92 ± 11.51[c]
LF (n=6)	315.40± 225.50[d]	147.53± 60.53[f]	255.63 ± 218.90[f]	10.31 ± 4.78[e]
LCBAT (n=6)	378.25± 191.31[e]	100.00± 26.39[d]	331.04± 191.52[e]	13.85 ± 6.67[g]

[a]$p < 0.001$ vs LMr, [b]$p < 0.05$ vs LMr, [c]$p = 0.01$ vs LMr,
[d]$p < 0.003$, [e]$p < 0.001$, [f]$p < 0.002$, [g]$p < 0.004$ vs LMA

TABLE no.7 – Influence of treatment with bioactive complex of triterpene acids (CBAT) and with Fluvastatin, respectively, on some biochemical blood parameters in rabbits fed with atherogenic diet for 42 days.

Significance of media difference by Student "t" – test at $p<0.05$

Group	Parameter		
	TGO (U/l)	TGP (U/l)	Creatine phosphokinase
LMr (n=6)	63.33 ± 19.86	86.57 ± 37.43	1678.33 ± 274.04
LMA (n=6)	59.53 ± 30.06	86.69 ± 35.27	1760.00 ± 1144.17
LF (n=6)	243.06 ± 152.52[a]	169.19 ± 38.77[a]	7997.50 ± 4660.74[a]
LCBAT (n=6)	64.4 ± 12.41	71.53 ± 23.96	2556.5 ± 941.4

[a]$P < 0.05$ vs LMr

From analysis of data shown in tables nos.5, 6, 7, the following can be concluded:

a. atherogenic diet daily administered for 42 days in rabbits from atheromatosis control group induces an advanced atheromatosis process at aorta level that is correlated with a significant increase of triglycerides, cholesterol, lipoproteins, LDL and dimension of cholesterol/HDL ratio;

b. bioactive complex of triterpene acids administered for 42 days in rabbits in a dose of 50 mg/kg b.w. per oral shows a significant antiatheromatous action,

evidenced by the size of score/group as compared with values corresponding to LMA atheromatosis control group;

c. Fluvastatin administered in a dose of 1.5 mg/kg b.w. per oral in rabbits, though having a significant antiatheromatous action, induces severe adverse effects, exhibited by:
   - significant increases of TGO, TGP transaminases and creatine phosphokinase;
   - continuous loss of body weight, in the last week getting a dystrophic appearance, with fur loss on extensive areas of the body;
   - in anatomopathological microscopic examination, colour, consistency and volume changes were revealed in the liver, accompanied by tumour formations of 4...6 mm diameter, too.

d. Under the same experimental conditions, treatment with bioactive complex of triterpene acids did not cause adverse hepatotoxic effects of Fluvastatin.

e. Experimental data obtained and shown in tables nos.5, 6, 7 demonstrate considerable hypolipidemic and antiatherogenous activity of bioactive complex of triterpene acids, which justifies its therapeutical use in the treatment of hyperlipidemies with high risk of coronary disease and other diabetes associated ones.

B. IMMUNOPROTECTIVE ACTIVITY OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS IN THE THERAPY WITH GLUCOCORTICOSTEROIDS AND CYTOSTATICS.

I. IMMUNOPROTECTIVE EFFECT IN THE THERAPY WITH GLUCOCORTICOSTEROIDS

Having in view that both the glucocorticosteroids therapy and a number of serious diseases (cancer, viroses) are characterized by a drastic lymphocyte depression, followed by lowering of body immune response, the influence of administration of bioactive complex of triterpene acids (CBAT) was followed on an experimental model adequate for immunobiological studies; the model consists in experimental induction of lymphocyte depression by administering a single dose of cortisone, 6 mg/100 g b.w., in white Wistar rats.

Subsequently, the animals were given bioactive complex of triterpene acids in doses of 50 and 100 mg/kg b.w. respectively, for 3 days. Experimental data are shown in tables nos. 8, 9, 10 where: LM=control group; LC=group treated with Cortisone –

acetate; LCCBAT 50 = group treated with Cortisone – acetate + CBAT 50 mg/kg b.w.; LCCBAT 100 = group treated with Cortisone – acetate + CBAT 100 mg/kg b.w.

Table no. 8 - Effect of treatment with bioactive concentrate of triterpene acids (CBAT) on thymus absolute and relative weight in Wistar rats after administration of Cortisone – acetate

Significance of media differences by Student "t" – test at $p < 0.05$

Group	Parameter			
	Thymus absolute weight, (mg)	Differen-ce against control group %	Thymus relative weight (mg/100 g body)	Differen ce against control group %
LM (n=16)	198.25 ± 4.98	-	145.60 ± 6.75	-
LC (n=20)	79.30 ± 6.45[a]	- 60	57.96 ± 6.03[a]	- 60.10
LCCBAT 50 (n=20)	130.48 ± 8.21[a,b]	- 34.18	89.93 ± 5.85[a,b]	- 40.15
LCCBAT 100 (n=20)	135.10 ± 9.05 [a,b]	- 31,85	93.60 ± 6.48 [a,b]	- 37.71

[a]$p < 0.001$ vs Control group, [b]$p < 0.001$ vs Cortisone – acetate

From the table one can conclude that, at the thymus level - a junction organ between neuroendocrine and immunological homeostasis of the body – the treatment with bioactive complex of triterpene acids largely counteracts thymus involution induced by cortisone – acetate, exhibited by drastic decrease of lymphocytes.

Experimental data shown in table 9 reveal decrease of total serum proteins after administration of Cortisone – acetate, the result achieved being in concordance with known adverse effects of corticosteroids on proteinic metabolism. Treatment with bioactive complex of triterpene acids makes the content of total serum proteins to be restored to the control group value.

Also, administration of Cortisone – acetate causes a decrease in liver proteins which is restored by treatment with bioactrive complex of triterpene acids, so that both groups record an increase of liver proteins by over 6% as against control group value and by 11.6%, as against the group treated with Cortisone-acetate.

TABLE no.9 – Effect of treatment with bioactive complex of triterpene acids (CBAT) on the content of total serum and liver proteins in Wistar rats after administration of Cortisone – acetate

Significance of media difference by Student "t" – test at $p<0.05$

Group	Parameter			
	Total serum proteins (g/100 ml serum)	Difference against control group %	Liver proteins (mg/100 g tissue)	Difference against control group %
LM (n=10)	8.67 ± 0.15	-	122.78 ± 2.8	-
LC (n=10)	7.73 ± 0.11[a]	- 10.84	117.65 ± 2.32	- 4.18
LCCBAT 50 (n=10)	8.57 ± 0.32	- 1.15	131.15 ± 4.07[c]	+ 6.82
LCCBAT 100 (n=10)	9.02 ± 0.14[b]	+ 4.04	131.31 ± 3.26[c]	+ 6.95

[a]p < 0.05 vs. Control group, [b]p < 0.001 vs. Cortisone-acetate, [c]p < 0.01 vs. Cortisone-acetate.

From the table, one can conclude that the treatment with bioactive complex of triterpene acids does not only restore decrease in number of leucocytes induced by administration of Cortisone – acetate, but even stimulate their number increase over control value.

TABLE no.10 – Effect of the treatment with bioactive complex of triterpene acids (CBAT) on number of leucocytes and serum gamma-globulins, in Wistar rats, after administration of Cortisone-acetate.

Significance of media difference by Student "t" – test at p <0.05

Group	Parameter			
	Leucocytes (cells/mm^3 blood)	Difference against control group (%)	Serum gamma-globulins (g/100 ml serum)	Difference against control group (%)
LM (n=10)	7907.5 ± 256.3	-	1.66 ± 0.14	-
LC (n=10)	5464 ± 601	- 30.9	1.45 ± 0.05	-12.65
LCCBAT 50 (n=10)	8484 ± 929.5[a]	+ 7.3	1.75 ± 0,06[c]	+ 5.42
LCCBAT 100 (n=10)	9120 ± 994.5[b]	+ 15.3	2.00 ± 0.09[c]	+ 20.48

[a]p<0.01 vs. Cortisone-acetate, [b]p<0.001 vs. control group and Cortisone-acetate, [c]p<0.01 vs. Cortisone-acetate.

From the table, one can also conclude that decrease in serum gamma-globulins after administration of Cortisone – acetate is restored by their return to a value higher

than that of control group in both groups treated with CBAT. Increase of gamma-globulin fraction shows CBAT stimulative effect in immunobiological processes of the body, simultaneously with significant antiimmunosuppressing effects in the therapy with glucocorticosteroids.

II. IMMUNOPROTECTIVE AND HEPATOPROTECTIVE EFFECT IN TREATMENT WITH CYTOSTATICS

Clinical oncological trials showed that cyclophosphamide and other cytostatics have a multitude of adverse effects, among which mention should be made of:

- myelodepression, hematopoiesis depression: leukopenia, thrombocytopenia;
- thymus involution, atrophy of adrenal glands and spleen;
- hepatotoxicity, due to metabolization at liver level;
- immunosuppressing and cytostatic effects at the level of body healthy tissues and organs;
- like other alkylating agents, cyclophosphamide has carcinogenic properties, with risk of developing a secondary tumour or acute leukaemia.

To evidence immunoprotective (antiimmunosuppressing) activity of bioactive complex of triterpene acids, an experimental model was used that recommended immunosuppression induction by cytostatic treatment in Wistar rats with Walker 256 tumour ascites caused by intraperitoneal administration of a tumour inoculum of 10^5 cells/animal. Animals were treated as follows:

LM = control group that was given physiological serum;

LCf = group with tumour inoculum of 10^5 cells/animal that was given cyclophosphamide in a dose of 15 mg/kg b.w. per oral/day;

LCfCBAT = group with tumour inoculum of 10^5 cells/ animal that was given cyclophosphamide in a dose of 15 mg/kg b.w. per oral/day and 100 g bioactive complex of triterpene acids/kg b.w. per oral/day.

Administration was started in 24 h after tumour cell inoculation and carried out throughout survival period of each animal of the groups treated as follows: after 21 treatment days, biochemical and haematological determinations were made and at the end of experiment, histopathological investigations were performed, the results shown in table no.11 being recorded.

From the table one can conclude that the pharmacological effect of treatment with bioactive complex of triterpene acids against the background of severe immunosuppression caused by cyclophosphamide, was a considerable increase in number of leucocytes in suppressed animals treated with bioactive complex of

triterpene acids as compared with those in the untreated group. Also, a significant increase is found in number of red cells, exceeding the value found in control group.

TABLE no.11 – Effect of treatment with bioactive complex of triterpene acids (CBAT) on the number of leucocytes and red cells in Wistar rats with Walker 256 tumour ascites induced by cyclophosphamide administration

Significance of media difference by Student "t" – test at $p< 0.05$

Group	Parameter			
	No. of leucocytes (cells/mm^3 blood)	Difference against control group (%)	No. of red cells (mil.cells/mm^3 blood)	Difference against control group (%)
LM (n=11)	8.977 ± 432	-	7.586 ± 0.169	-
LCf (n=12)	2.143 ± 203[a]	- 76.12	6.525 ± 0.141[a]	- 13.98
LCfCBAT 100 (n=12)	3.915 ± 386[a,b]	- 56.38	7.936 ± 0.271[c]	+ 4.6

[a]$p<0.001$ vs. control group, [b]$p<0.01$ vs. cyclophosphamide,

[c]$p<0.001$ vs. cyclophosphamide.

Due to metabolization at liver level, treatment with cyclophosphamide induced hepatotoxic effects too, exhibited by considerable increases in all plasma biochemical parameters investigated, as compared with values in control group. Association of bioactive complex of triterpene acids in a dose of 100 mg/kg b.w. per oral in the treatment with cyclophosphamide 15 mg/kg b.w. per oral in Walker 256 tumour ascites, keeps biochemical parameters investigated at values close to average values in control group, as resulted from table no.12.

Hepatoprotective activity evidenced by experimental biochemical data from table no.12 is in concordance with results of ultrastructural histopathological investigations, achieved within evaluation of influence of bioactive complex of triterpene acids on adverse hepatotoxic effects of cyclophosphamide, namely:

- following treatment of Wistar rats with cyclophosphamide, hepatocytes show a lipid metabolism disturbance, the liver cells nuclei undergoing an increase to a double volume against control group and nucleols ultrastructure is altered, swowing affection of nucleic acids synthesis and of cell proteins biosynthesis;
- treatment with bioactive complex of triterpene acids reduces alterations induced by cyclophosphamide in structure of hepatocytes and in their metabolic activity, thus the

cyclophosphamide effect of lipid accumulation in hepatocytes is annihilated, the protective action of bioactive complex of triterpene acids determining cyclophosphamide metabolization without producing structural alterations;

- bioactive complex of triterpene acids also counteracts cyclophosphamide effect of inhibiting nucleic acids synthesis, the presence of polyribosomes showing an increase in structural protein synthesis.

TABLE no.12 – Hepatoprotective effect of association of bioactive complex of triterpene acids (CBAT) with cyclophosphamide on some blood biochemical parameters in Walker 256 ascites in rats, compared with cyclophosphamide

Significance of media difference by Student "t" – test at $p < 0.05$

Group	Biochemical parameters				
	GOT (U/l)	GPT (U/l)	γ-GT (U/l)	Alkaline phosphatase (U/l)	Bilirubine-mia (mg/dl)
LM (n=6)	191.33±34.94	27.81±5.66	7.00± 1.41	485.83± 50.19	0.45 ± 0.04
LCf (n=6)	269.58±91.77[a]	37.50±4.23[a]	5.72±7.30[b]	828.17±155.17[c]	0.63±0.07[c]
LCfC BAT (n=6)	168.50±41.44[d]	26.01± 6.03[c]	5.60 ±1.86[f]	467.00±51.94[e]	0.47± 0.02[g]

[a]$p < 0.05$; [b]$p < 0.002$; [c]$p < 0.003$ vs Control group

[d]$p < 0.05$; [e]$p < 0.01$; [f]$p < 0.001$; [g]$p < 0.003$ vs Cyclophosphamide

Comparative experimental data show that, bioactive complex of triterpene acids concomitantly administrered with cyclophosphamide counteracts its hepatotoxic and immunosuppressing adverse effects, which motivates bioproduct application as hepatoprotector and immunosuppressor in oncological therapy with cytostatics.

C. INFLUENCING ACTIVITY OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS ON TUMOUR PROGRESSION EVOLUTION

In the experimental model of Walker 256 ascitic cancerigenic tumour induced in Wistar rats, the bioactive complex of triterpene acids proved to retard tumour progression.

Experimental data obtained are shown in table no.13.

TABLE no.13 – Influence of treatment with bioactive complex of triterpene acids (CBAT) on latency period until cancerous tumour occurrence and average survival time

Significance of media difference of Student "t" – test at $p < 0.05$

Tested group	Parameters	
	Latency period until tumour occurrence (days/group)	Average survival time (days/group)
LMT (n=10)	7.20 ± 0.42	22.00 ± 6.04
LTCBAT (n=10)	8.90 ± 1.66[a]	24.60 ± 5.83[b]

[a]$p = 0.002$, [b]$p < 0.001$ vs. control group

LMT = control group with tumour;

LTCBAT = group with tumour + CBAT 100 mg/kg b.w.

The carcinogenic potential of Walker 256 tumour line used to induce ascitic cancerous tumour and also tumour inoculum size were appropriate, since tumour incidence in the group used as control group inoculated with 10^5 cells/animal, is 100%.

From the table, one can find out that, bioactive complex of triterpene acids, in a dose of 100 mg kg/b.w. per oral in Wistar rats inoculated with 10^5 cells/animal produced significant extensions of latency period from inoculation until cancerous tumour occurrence and of average survival time, compared with latency period from inoculation until cancerous tumour occurrence and average survival time in control group, inoculated under the same conditions.

D. LOCAL ANTIINFLAMMATORY ACTIVITY OF BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Evaluation of local antiinflammatory activity of bioactive complex of triterpene acids (CBAT), as compared with Indomethacin, was carried out on mice that were induced an inflammation at the ear level with TPA (12-0-tetradecanoylphorbol acetate), within two types of experiments:

1. acute experiment: immediately after application of a dose of TPA (2.5μg/ear) on both front sides of the ears, CBAT was applied on the right ear (in different doses); after 4 hours animals were killed and 6 mm diameter-circular biopsies were collected from each ear, that were weighed at once;
2. chronic experiment: was carried out on a 10 days – period; TPA administration was made on days 1, 3, 5, 8, 9 and 10, and CBAT was given on days 8, 9, 10 at 10.00 o'clock a.m simultaneously with TPA administration and at 4.00 o'clock p.m.; animals

were killed at the end of experiment and 6 mm diameter – circular biopsies were collected from each ear, which were weighed.

Results of the two experiments are shown in table no.14.

Experimental data obtained within the two experiments applied and shown in table no.14 reveal that, bioactive complex of triterpene acids has a statistically significant local antiinflammatory activity, acting as an antiphlogistic agent that inhibits exudative inflammatory and proliferative processes.

Activity of bioactive complex of triterpene acids (CBAT) in a dose of 0.4μmol/cm^2 is comparable with that one of Indomethacin – used as a reference antiinflammatory medicinal drug and tested under the same experimental conditions.

TABLE no.14 – Local antiinflammatory activity of bioactive complex of triterpene acids (CBAT), as compared with Indomethacin

Significance of media difference by Student "t' – test at $p < 0.05$

Group	Acute experiment			Chronic experiment		
	Biopsy fragment mass (mg)		Anti-inflam-matory activity %	Biopsy fragment mass (mg)		Anti-inflammatory activity (%)
	l.e.	r.e.	-	l.e.	r.e.	-
LM (n=10)	8.3 ± 0.5		-	8.3 ± 0.5		-
LTC (n=10)	16.31± 2.1	10.41±1.6[a]	73.66	18.32 ± 0.6	11.83± 0.8[a]	64.77
LTC2 (n=10)	14.76 ± 1.5	10.63± 1.3[a]	63.93	17.47 ± 1.9	12.44± 0.7[a]	54.85
LTC3 (n=10)	15.02 ± 1.7	12.06±1.6[b]	44.9	16.45 ± 2.1	13.5± 1.1[c]	36.2
LTI (n=10)	17.79 ± 1.9	11.11± 0.8[a]	70.35	16.6 ± 1.1	10.8± 0.4[a]	69.8

l.e. = left ear (inflammation control group); r.e. = right ear;

LM = absolute control group; LTC1 = group with TPA + CBAT 0.4 μmoli/cm^2 ear; LTC2 = group with TPA + CBAT 0.25 μmoli/cm^2 ear; LTC3 = group with TPA + CBAT 0,1 μmoli/cm^2 ear; LTI = group with TPA + Indomethacin 0.4 μmoli/cm^2 ear;

[a]$p < 0.00001$; [b]$p < 0.05$; [c]$p<0.005$ vs. corresponding control groups

Results achieved motivates therapeutical use of bioactive complex of triterpene acids in pharmaceutical formulas for external use intended for local treatment of different inflammatory processes, such as: inflammatory and pruriginous dermatoses, eschars, burns, psoriasis, postoperative, post-traumatic and rheumatic inflammatory conditions.

EXAMPLES OF CONDITIONING THE BIOACTIVE COMPLEX OF TRITERPENE ACIDS IN THE FORM OF MEDICINAL PRODUCTS

Based on the results of pharmacological tests performed, bioactive complex of triterpene acids as such or in the form of Na, K, or NH_4 salts, can be conditioned in the form of medicinal products for internal or external use, meant for clinical fields with large social incidence belonging to human or veterinary therapeutics. Medicinal products described further, according to invention, are administered in human or veterinary therapeutics, by injection, orally or locally, for 1 day – 24 months, depending on disease progression, in daily doses of 10-1000 mg/kg b.w. divided in 1-6 intakes. Formulation examples shown further on do not preclude possible development of other conditioning the formulas, considering diversity and multitude of excipients known and used in pharmaceutical industry.

Example no.8

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF INJECTABLE SOLUTION CONTAINING 2% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids - Na salt min.90%	21 g
- benzyl alcohol	100 ml
- phosphate buffer aqueous solution pH=8	100 ml
- propylene glycol	add.1000 ml

Method for preparation: powder of triterpene acids - Na salt is hot dissolved (60...70^{0}C) in 200 ml mixture of 1/1 v/v benzyl alcohol: propylene glycol, the remaining propylene glycol, and phosphate buffer aqueous solution pH=8 are added and the resulted clear solution is aseptically filtered and conditioned in 1 ml or 2 ml ampoules containing 20 mg/ml bioactive complex of triterpene acids – Na salt.

The product is curatively given, in doses of 1...3 ampoules/day, for 1...6 months, having the following pharmacological effects: antitumorigenic, immunoprotective, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonific, especially at coronary level, in the following diseases:

neoplasm, acute and chronic hepatites, liver cirrhosis, immune system depletions occurred after treatment with glucocorticosteroids, cytostatics.

Example no.9

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF PERFUSABLE EMULSION CONTAINING 1% BIOACTIVE COMPLEX OF TRITEPRPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids – Na salt min.90%	10.5 g
- refined soy oil	100 g
- egg phospholipids	12 g
- anhydrous glycerine	25 g
- vitamin E	1 g
- natriumoleate	0.3 g
- alkalinized distilled water for injectables with 0.003 g NaOH at pH=8	add. 1000 ml

Method for preparation: soy bean oil is heated at 70^0C, in which the bioactive complex of triterpene acids – Na salt is dissolved, and egg phospholipids, vitamin E and natrium oleate are added in the resulted solution. In distilled water for injectables, alkalinized with 0.003 g NaOH at pH=8, anhydrous glycerine is added; the two phases-hydrophilic and lipophilic – are homogenized in a homogenizer, at 80...90^0C at a pressure of 125...220 kg/cm^2, under nitrogen atmosphere until dispersion of lipophilic phase, whose drops should reach an average diameter of 0.5μm. Emulsion viscosity is ranged between 80...125 mPa x s and it is aseptically conditioned in sterilized glass vials under nitrogen atmosphere. Perfusable emulsion such prepared contains 10 mg/ml bioactive complex of triterpene acids and is administered in doses of 60...100 ml/day, having the following pharmacological effects: antitumorigenic, immunoprotective, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic particularly at coronary level with therapeutical uses, especially in clinical cases where there are not possible oral administration and enteric absorption, as follows: neoplasm, III-rd - IV-th stages, severe depletions of immune system, shocking conditions: serious burns on large areas of the body, hepatic or postoperative coma.

Example no.10

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF BUVABLE

SOLUTION CONTAINING 2% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids - Na salt min.90%	21 g
- ethyl alcohol 95^{C}	200 ml
- phosphate buffer aqueous solution pH=8	200 ml
- p-methyl hydroxybenzoate	1 g
- p-propyl hydroxybenzoate	2 g
- glycerin	300 ml
- propylene glycol	add.1000 ml

Method for preparation: a mixture of 200 ml ethyl alcohol 95^{C}, 300 ml glycerin and 200 ml propylene glycol is prepared, in which 21 g bioactive complex of triterpene acids – Na salt are hot dissolved (60...70^{0}C) under stirring. 200 ml phosphate buffer aqueous solution with pH=8 and the remaining propylene glycol, in which the two preservatives provided in the formula were dissolved, are added, then stirred up for homogenization, then filtered, and the resulted clear solution is conditioned in vials of 100 ml each.

The product contains 20 mg/ml bioactive complex of triterpene acids in the form of Na salt and is administered in doses of 15...20 ml/day, for 1...6 months, for preventive or curative purpose, especially in pediatrics, having the following pharmacological effects: antitumorigenic, antiimmunosuppressive, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic, especially at coronary level with therapeutical uses in the following diseases: neoplasm, acute and chronic hepatites, immune system depletions occurred following treatment with glucocorticosteroids and cytostatics, postoperative and post-traumatic conditions.

Example no.11

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF CAPSULES CONTAINING 100 mg BIOACTIVE COMPLEX OF TRITERPENE ACIDS/CAPSULE

Conditioning formula/capsule

- bioactive complex of triterpene acids – min.90%	105 mg
- lactose monohydrate	150 mg
- starch sodium glycolate	9.0 mg
- polyvinylpyrolidone K30	6.5 mg

- sodium lauryl sulphate 2.0 mg
- aerosil 1.47 mg
- talc 7.25 mg
- magnesium stearate 2. 9 mg

Method for preparation: bioactive complex of triterpene acids is sieved with the excipients: lactose monohydrate and aerosil on a II-sized sieve. A solution made by dissolving polyvinylpyrolidone K30 in ethyl alcohol is poured over the obtained mixture and then it is homogenized. Granules are produced that are dried at temperature of 35...45^{0}C and then are ground to the size V given by sieve; starch sodium glycolate, sodium lauryl sulphate, talc and magnesium stearate are then added, homogenized and the powder produced is placed in 1 sized capsules. Product is administered in doses of 3...6 capsules/day, for 1...24 months, having the following pharmacological effects: antitumorigenic, immunoprotective, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic especially at coronary level, with therapeutic uses in the following diseases: neoplasm, immune system depletions, acute and chronic hepatites, hepatic cirrhosis, ulcerous disease, diabetes, cardiovascular disorders of atherosclerotic etiology, vascular, tissular and dermic collagen degenerations: psoriasis, neurodermatitides, radiodermatitides, varicous syndrome and different symptoms of chronic venous insufficiency, vascular retinal affections, applicable in geriatrics too, for preventing and slowing down ageing processes.

Example no.12

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF TABLETS CONTAINING 100 mg BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Conditioning formula/tablet:

- bioactive complex of triterpene acids – min.90% 105.00 mg
- lactose monohydrate 81.20 mg
- microcrystalline cellulose 74.00 mg
- corn starch 16.20 mg
- starch sodium glycolate 5.00 mg
- polividone 5.60 mg
- magnesium stearate 2.00 mg
- aerosil 1.00 mg
- talc 5.00 mg

Method for preparation: the usual known conditioning procedures are applied for pharmaceuticals for internal use in the form of tablets on industrial facilities, complying with good manufacturing practice regulations for medicinal drugs stipulated by the European legislation.

The product is administered in doses of 3...6 tablets/day for 1...24 months, having the following pharmacological effects: antitumorigenic, immunoprotective, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic, especially at coronary level, with therapeutic uses in the following diseases: neoplasm, immune system depletions, acute and chronic hepatites, hepatic cirrhosis, ulcerous disease, diabetes, cardiovascular disorders of atherosclerotic etiology, vascular, articular and dermic collagen degenerations: psoriasis, neurodermatitides, radiodermatitides, varicous syndrome and different symptoms of chronic venous insufficiency, vascular retinal affections, applicable in geriatrics too, for preventing and slowing down ageing processes.

Example no.13

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF EYE WASH CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids - Na salt min.90%	10.5 g
- benzalkonium chloride	0.1 g
- refined castor-oil	add.1000 g

Method for preparation: 0.1g benzalkonium chloride and 10.5 bioactive complex of triterpene acids – Na salt are aseptically dissolved by heating at 70^0C, and the resulted clear solution is aseptically filtered and conditioned in vials of 10 ml each, with eye dropper.

The product contains 10 mg/ml bioactive complex of triterpene acids in the form of Na salt and is administered for preventive or curative purpose, in doses of 0.2...0.5 ml/day, for 1...6 months, having the following pharmacological effects: trophic and regenerative for retinal vascular and tissular collagen, antiinflammatory, with ophthalmological therapeutic uses: diabetic retinopathy, cataract, postoperative treatment of cataract, glaucoma and cornea transplant.

Example no.14

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF OPHTHALMIC OINTMENT CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids - Na salt min.90%	10.5 g
- emulsifying cetyl stearyl alcohol	300 g
- white vaseline	350 g
- benzalkonium chloride	0.1 g
- paraffin oil	add.1000 g

Method for preparation: 0.1 g bezalkonium chloride and 10 g bioactive complex of triterpene acids – Na salt are aseptically dissolved by heating at 70^0C, resulting in a slightly opalescent solution, which is incorporated into the mixture melted at 60^0C, consisting of: 300 g emulsifying cetyl stearyl alcohol, 350 g white vaseline with the remaining paraffin oil, and then it is triturated until cooling. An ophthalmic ointment is obtained, in the form of a buttery, homogeneous, white mass, containing 1% bioactive complex of triterpene acids – Na salt, which is aseptically conditioned in tubes of 10 g each.

Due to its pharmacological restoring effects on vascular and dermic collagen, it is used in local ophthalmologic therapy, in doses of 10...20 mg/day, for 1...6 months, having the following pharmacological effects: trophic and regenerative for retinal vascular and tissular collagen, antiinflammatory, with therapeutical ophthalmologic uses: diabetic retinopathy, cataract, postoperative treatment of cataract, glaucoma and cornea transplant

Example no.15

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF GEL-FOR DERMATOLOGICAL USE, CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids – Na salt min.90%	10.5 g
- Carbopol 940	20 g
- glycerin	60 g
- 15% sodium hydroxide solution	10 g
- prolylene glycol	60 g
- p-methyl hydroxybenzoate	1 g
- p-propyl hydroxybenzoate	2 g
- "I" structured water (pH=1.8...2.6)	add. 1000 g

Method for preparation: gel mass is prepared by dispersion of 20 g Carbopol 940 in the mixture consisting of 60 g glycerin with 837 g "I" structured water in which the

mixture of preservatives mentioned in the formula is dissolved. It is gently stirred and left at rest at 15...25^{0}C, afterwards the mixture composed of 10.5 g bioactive complex of triterpene acids, 60 g propylene glycol and 10 g of 15% sodium hydroxide solution is added under slow stirring, pH is adjusted to nearly 7.0 and stirring is continued for 30 minutes in order to become well homogenized and then it is packaged in tubes of 50 g each.

The product contains 1% bioactive complex of triterpene acids – Na salt and is administered in doses of 10...30 mg/day, for 1...6 months, having the following pharmacological effects: trophic and regenerative for vascular, tissular, articular and dermic collagen, antiinflammatory, antihyaluronidase and antielastase, with therapeutic uses in the following diseases: burns, post-burn and postoperative cheloids, eschars, atonic wounds of different etiologies, degenerations of dermic, vascular, tissular and articular collagen such as: skin benign and malignant diseases, psoriasis, inflammatory and pruriginous dermatoses, neurodermatitides, radiodermatitides, variceal and post-thrombotic syndrome, chronic venous insufficiency, vascular fragility, rheumatic inflammatory conditions, and diabetic or endocrine obesity as well.

Example no.16

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF CREAM CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula:

- bioactive complex of triterpene acids - Na salt min.90%	10.5 g
- cetyl alcohol	200 g
- white vaseline	200 g
- glycerin	120 g
- Tween 80	70 g
- p-methyl hydroxybenzoate	1 g
- p-propyl hydroxybenzoate	2 g
- "I" structured water (pH=1.8...2.6)	add.1000 g

Method for preparation: 10.5 g bioactive complex of triterpene acids – Na salt are dissolved in 120 g glycerine and the resulted solution is incorporated into the ointment mass produced by melting cetyl alcohol and white vaseline at 60^{0}C, followed by emulsification by adding 70 g Tween 80 and minimum 400 g "I" structured water in which the preservatives specified in the formula were dissolved beforehand. The ointment mass is stirred for emulsification and homogenization, and afterwards it is packaged in tubes of 50 g each.

The product is in the form of a white, homogeneous, unctuous, rapidly absorbable cream, containing 1% bioactive complex of triterpene acids as Na salt, and it is administered in doses of 10...30 mg/day, for 1...6 months, having the following pharmacological effects: trophic and regenerative for vascular, tissular, articular and dermic collagen, antiinflammatory, antihyaluronidase and antielastase, with therapeutic uses in the following diseases: burns, post-burn and postoperative cheloids, eschars, atonic wounds of different etiologies, degenerations of dermic, vascular, tissular and articular collagen such as: skin benign and malignant affections, psoriasis, inflammatory and pruriginous dermatoses, neurodermatitides, radiodermatitides, variceal and post-thrombotic syndrome, chronic venous insufficiency, vascular fragility, rheumatic inflammatory conditions and diabetic or endocrine obesity, as well.

Example no.17

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF OINTMENT CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning formula

- bioactive complex of triterpene acids - Na salt min.90%	10.5 g
- propylene glycol	100 g
- benzalkonium chloride	0.1 g
- polyethylene glycol 4000	300 g
- polyethylene glycol 400	add.1000 g

Method for preparation: 10.5 g bioactive complex of triterpene acids – Na salt and 0.1 g benzalkonium chloride are dissolved in 100 g propylene glycol by heating at 60...70^{0}C and the resulted solution is incorporated, after cooling, into the ointment mass resulted from melting of the mixture of 300 g polyethylene glycol 4000 and 590 g polyethylene glycol 400 at 65...70^{0}C. It is mixed by gentle stirring, a white, homogeneous, unctuous mass being produced that contains 1% complex of bioactive triterpene acids – Na salt and is packaged in tubes of 50 g each.

The product is administered in doses of 10...30 mg/day for 1...6 months, having the following pharmacological effects: trophic and regenerative for vascular, articular and dermic collagen, antiinflammatory, antihyaluronidase and antielastase, with therapeutic uses in the following diseases: burns, post-burn and postoperative cheloids, eschars, atonic wounds of different etiologies, degenerations of dermic, vascular, tissular and articular collagen such as: benign and malignant skin affections, psoriasis, inflammatory and pruriginous dermatoses, neurodermatitides, radiodermatitides,

varicous and post-thrombotic syndrome, chronic venous insufficiency, vascular fragility, rheumatic inflammatory conditions, and diabetic or endocrine obesity as well.

Example no.18

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF SOLUTION FOR EXTERNAL USE OR SPRAY CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS – Na Salt

Conditioning form:

- bioactive complex of triterpene acids – Na salt min.90% 1.05 g
- isopropyl alcohol 50 g
- propylene glycol add.1000 g

Method for preparation: the mixture of isopropyl alcohol and propylene glycol is prepared in which the bioactive complex of triterpene acids is dissolved by heating at 70...80^{0}C and the resulted solution is packaged in spray vials of 100 ml each or in Freon pressurized vials.

The product is administered in doses of 1...2 g/day, for 1...3 months, having the following pharmacological effects: trophic and regenerative for dermic, vascular and tissular collagen, membrane fluidity modulator, with therapeutic uses in the following diseases: burns, eschars, atonic wounds of different etiologies, allergic, inflammatory and pruriginous dermatoses, including psoriasis and radiodermatitides.

Example no.19

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF SUPPOSITORIES CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Conditioning formula:

- bioactive complex of triterpene acids – min.90% 1.05 g
- polyethylene glycol 400 5 g
- para-methyl hydroxybenzoate 0.1 g
- para-propyl hydroxybenzoate 0.03 g
- Suppocire (a mixture of half-synthesis triglycerides) add.100 g

Method for preparation: the bioactive complex of triterpene acids is dissolved in polyethylene glycol by heating at 80^{0}C. The resulted solution is cooled at 40^{0}C and it is added over Suppocire mass heated beforehand at 40^{0}C. The mixture of preservatives is added, then homogenized and poured in suppository forms weighing 2.5 g each.

The product contains 25 mg bioactive complex of triterpene acids/suppository and it may be administered in doses of 50...100 mg/day for 1...6 months having the following pharmacological effects: antitumorigenic, immunoprotective, anti-inflammatory,

trophic and regenerative for vascular, tissular and articular collagen, with pharmacological uses in the following diseases: neoplasm particularly in the colon, immunosuppressive conditions especially hepatotoxic ones, occurred after the treatment with glucocorticosteroids, cytostatics, rheumatic inflammatory diseases, haemorrhoidal syndrome.

Example no.20

MEDICINAL PRODUCT CONDITIONED IN THE FORM OF OVULES CONTAINING 1% BIOACTIVE COMPLEX OF TRITERPENE ACIDS

Conditioning formula:

- bioactive complex of triterpene acids – min.90%	1.05 g
- polyethylene glycol 400	5 g
- para-methyl hydrobenzoate	0.1 g
- para-propyl hydrobenzoate	0.3 g
- Ovucire (mixture of half-synthesis triglycerides)	add.100 g

Method for preparation: the bioactive complex of triterpene acids is dissolved in polyethylene glycol, by heating at 80^0C. The resulted solution is cooled at 40^0C and added over Ovucire mass heated beforehand at 40^0C. The mixture of preservatives is added, then homogenized and poured in ovule forms weighing 2.5 g each.

The product contains 25 mg bioactive complex of triterpene acids/ovule and may be given in doses of 25...100mg / day for 1...6 months, having the following pharmacological effects: antitumorigenic, antiinflammatory, trophic and regenerative for dermic and tissular collagen, with the following therapeutic uses: benign or malignant tumorigenic diseases at the level of vagina and uterine cervix, trophic vulvovaginal disorders and cervicites, particularly in the menopause.

"I" – inhibitively activated and "S" – stimulatively activated structured waters are produced (Patents of Ioan MANZATU et al. nos. RO 109835/1996, US 5846397/1998, EP 0777631/1999, CN/ZL 951914722.4/2003) by passing a demineralised or distilled water, enriched - under control - in mineral salts, in order to reach conductivity values of 300...500μS, in a chemically neutral, parallelipipedal column, with several structuring cells consisting of activators with two lamellar electrodes each, one negative and the other one positive, made up of stainless steel net, placed on one and the other side of a sandwich of porous, chemically inert membranes and, following the interaction processes between the water dipolar molecular structures and electrostatic field generated between electrodes, a process occurs by which there are produced arrangements, polarizations and energy needed to bind by hydrogen bridges the water

molecules and negative radicals [R^-], resulting in "I"-inhibitively activated structured water, with pH of 1.8...2.6 and conductivity of 1200...2500μS, discharged from the left-hand and respectively, right-hand spaces of the positive electrodes of each activator, and simultaneously, in the spaces between negative electrodes, there are produced arrangements, polarizations and required energy for binding by H and HO bridges the water molecules in polymolecular

aggregates with [R^+] positive radicals, resulting in "S" – stimulatively activated structured water with pH of 10...12 and conductivity of 800...1300μS.

INDUSTRIAL APPLICABILITY

The technological process making the subject matter of invention is applicable at industrial scale, being reproducible on the usual instrumentation and equipment existing in the industry of plant extractions.

BIBLIOGRAPHY

1. D.BARICEVIC	Journal of Ethnopharmacology , 72(2001), 125-132
2. Paik KEE-JOO et al.	Arch.Pharmacology Research 1998, 21(4), 398-405, Chemical Abstracts vol. 129, 1998 (258259 b)
3. RINGBORN Therese et al.	Journal National Products, 1998, 61(10), 1212-1215- Chemical Abstracts, vol.129, 1998 (239627 d)
4. Es-SAADY D. et al.	Mediaters Inflammation 1994, 3(3), 181-184, Chemical Abstracts vol.121, 1994 (194953 y)
5. A. NAJID et al.	Federation of European Biochemical Societies vo.299, no.3, 213-217, 1992
6. Patent	GR 10011738 / 07.06.1993
7. Patent	US 6740778/25.05.2004
8. Patent	SU 18186346 / 3 / 27.09.1995
9. Patent	RU 2108107 / 10.04.1998
10. Patent	RU 2108803 / 20.04.1998
11. Patent	RU 2151139 / 20.06.2000
12. Patent	EP 1161879 / 12.12.2001
13. Patent application	US 2002037882 /28.03.2002
14. Patent	EP 1250852/23.10.2002
15. Patent application	US 2003049365/13.03.2003
16. Patent	CN 1358733/17.07.2002
17. Jie Liu	Journal of Ethnopharmacology, 49(1995), 57-68
18. Samova L.I., Shode F.O. et al.	Journal of Ethnopharmacology, 2003 Feb, 84(2-3): 299-305
19. Somova L.I. Shode F.O.	International Journal of Phytotherapy&Phytopharm 3/1/2003
20. Kuttan G. et al.	International Journal of Phytotherapy&Phytopharm 7/1/2003
21. Patent	US 4752606/1998
22. Patent apllication	US 10/488682/2002
23. Patent application	JP 05-211065
24. Patent application	EP 1495754 A2
25. Patent application	EP 0943620 A2

26. Patent US 5985924/1999
27. Patent application JP 63166195
28. Patent application JP 09-199323
29. Patent US 485754/1989
30. Jhonson R. Nature, 1972, 240, 412
31. Doug, Xoaping et al Yaown Feuxi Zazhi 1985, 5(1) 45-46 (CH).
32. Patent RO 109834/1996 – Ioan MANZATU et al.
33. Patent US 5846397/ 1998 – Ioan MĂNZATU et al.
34. Patent EP 0777631/ - Ioan MANZATU et al.
35. Patent CN/ZL 951914722.4/2003 – Ioan MANZATU et al.

CLAIMS

1. Bioactive complex of triterpene acids characterized in that it has a minimum 90% content, represented by: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids such as: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic obtained in the form of free acids or as Na, K, NH_4 salts, by extraction from the following plant species: *Salvia species, Lavandula species, Sambucus nigra and Crataegus species*, showing the following pharmacological effects:

- bioregulating activity on proteinic, glucidic and lipidic metabolism, particularly on aged organisms, with applications in the preventive and curative therapy of metabolic and cardiovascular diseases of atherosclerotic etiology, especially in geriatrics;
- immunoprotective and hepatoprotective activity of counteracting immuno-suppressive and hepatotoxic adverse effects occurred after treatment with glucocorticosteroids and cytostatics;
- retarding activity in tumour progression;
- antioxidizing activity;
- local antiinflammatory activity.

2. Bioactive complex of triterpene acids, according to claim 1, characterized in that, in order to increase product biovailability and solubility, in some conditioning solvents, it is also produced and used in the form of Na, K, or NH_4 salts of minimum 90% purity, consisting of: minimum 75% Na or K or NH_4 ursolate, 10...15% Na or K or NH_4 oleanolate and 4...10% Na, K or NH_4 salts of other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.

3. Bioactive complex of triterpene acids, according to claim 1, characterized in that it is obtained from the following plant raw materials:

- *herba Salvia species: Salvia officinalis, Salvia lavandufolia, Salvia triloba, Salvia sclarea* etc.;
- *herba Lavandula species: Lavandula angustifolia, Lavandula vera, Lavandula spica, Lavandula fragrans, Lavandula latifolia,* etc.
- lavender residue, resulted from production of essential oil by steaming;
- flowers of leaves of *Sambucus nigra*;
- leaves and flowers of *herba Crataegus species: Crataegus monogyna and Crataegus oxyacantha.*

4. Production process for bioactive complex of triterpene acids, described in claim 1, characterized in that the dried and pulverized plant raw material, with 2...4% triterpene acids, consisting of *Salvia species herba, Lavandula species herba* - that can be not only the herba plant but also the resulted residue from production of essential oil by steaming, *Sambucus nigra-flowers* or leaves or *Crataegus species herba*, is extracted by dynamic maceration in the 1/15...1/30 m/v ratio, plant mass/solvent, with one of the extraction solvents represented by mixtures composed of: 5...15% "I" structured water with 85...95% acetone or with 85...95% ethyl alcohol 95^C, for 8...24 h, at 15...80^0C, the extractive solution being produced that, with a view to being purified, is passed on a chromatographic column with acid pH active granulated carbon, then it is vacuum concentrated at 35...40^0C up to 1/10...1/20 of the initial volume, a suspension being produced that is kept at 5...10^0C for 12...24 h, next it is vacuum filtered, the substance resulted after drying at 105^0C for 3 h, constituting the crude bioactive complex of triterpene acids with a minimum 70% content which, for purification, is reflux dissolved at 80^0C in the 1/100....1/200 m/v ratio in one of the solvents represented by the mixture consisting of: 5...10% "I" or "S" structured water with 90...95% ethyl alcohol 95^C or with 90...95% acetone; the resulted solution is purified by adsorption on acid pH active carbon added in the 0.1...0.3% m/v ratio, then it is filtered, vacuum concentrated at 35...40^0C up to 1/10...1/20 of the initial volume, the resulted microcrystalline suspension is kept for 12...24 h at 5...10^0C for finishing crystallization, next it is vacuum filtered, the precipitate produced is washed with 0.3...0.5 l distilled water, up to neutral pH, dried at 105^0C for at least 3 h and pulverized, resulting in crystallized bioactive complex of triterpene acids obtained by 1...2 crystallizations depending on the type of plant raw material processed at the same parameters, excepting the second concentration carried out in 1/5 of the initial volume, with a minimum 90% content represented by: minimum 75% ursolic acid, 10...15% oleanolic acid and 4...10% other triterpene acids consisting of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.

5. Process, according to claim 3, characterized in that, with a view to producing bioactive complex of triterpene acids in the form of Na, K and NH_4 salts, described in claim 2, the crystallized bioactive complex of triterpene acids with a minimum 90% content is reflux dissolved at 80^0C in the 1/100...1/200 m/v ratio in the alkaline pH solvent mixture: 90...95% ethyl alcohol 95^C with 5...10% "S" structured water, with pH=10...12, that also contains 1% NaOH or 1% KOH or 5% NH_4OH 25%, the solution produced is vacuum concentrated at 35...40^0C up to 1/5...1/10 of the initial volume, the

resulted suspension is kept for 12...24 h at 5...10^{0}C for finishing crystallization, followed by vacuum filtering, precipitate washing with 0.1...0.3 l distilled water up to neutral pH, drying at 105^{0}C for 3 h and pulverizing, the crystallized bioactive complex of triterpene acids being produced as Na or K or NH_4 salt, with a minimum 90% content consisting of: minimum 75% Na or K or NH_4 ursolate, 10...15% Na or K or NH4 oleanolate and 4...10% Na, K or NH_4 salts or other triterpene acids composed of one or more of the following acids: hydroxyursolic, hydroxyoleanolic, betulinic, dehydrobetulinic.

6. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids according claims 1 and 2, conditioned as injectable solution or perfursable emulsion or buvable solution for pediatric use, containing 1...2% bioactive complex of triterpene acids as such or in the form of salts, having the following pharmacological effects: antitumorigenic, immunoprotective, antioxidizing, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic, particularly at coronary level, being prevalently applicable in the following diseases: neoplasm, acute and chronic hepatitis, hepatic cirrhosis, immune system depletions, occurred following the treatment with glucocorticosteroids, cytostatics, rheumatic inflammatory or degenerative affections.

7. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids according to claims 1 and 2, conditioned as tablets or hard and/or soft jelly operculated capsules containing 50...100 mg bioactive complex of triterpene acids/capsule, having the following pharmacological effects: antitumorigenic, immunoprotective, antioxidizing, hepatoprotective, antiulcerous, hypoglycemic, hypolipidemic, antiatherosclerotic, antiinflammatory, vascular tonic especially at coronary level, with therapeutical use in the following diseases: neoplasm, immune system depletions, acute and chronic hepatites, hepatic cirrhosis, ulcerous disease, diabetes, cardiovascular disturbances of atherosclerotic etiology, degenerations of vascular, tissular and dermic collagen: psoriasis, neurodermatitides, radiodermatitides, varicous syndrome and different symptoms of chronic venous insufficiency, retinal vascular affections, applicable in geriatrics too, for preventing and slowing down ageing processes.

8. Medicinal product characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids according to claims 1 and 2, conditioned as eyewash or ophthalmic ointment containing 0.5...1% bioactive complex triterpene acids, as such or in the form of salts, having the following pharmacological

effects: trophic and regenerative for retinal vascular and tissular collagen, antiinflammatory, with therapeutical ophthalmological uses: diabetic retinopathy, cataract, in postoperative treatment of cataract, glaucoma and cornea transplant.

9. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutical active substance the bioactive complex of triterpene acids according to claims 1 and 2, conditioned as gel or cream or ointment containing 0.1...1% bioactive complex of triterpene acids as such or in the form of salts, having the following pharmacological effects: trophic and regenerative for vascular, tissular, articular and dermic collagen, antiinflammatory, antioxidizing, antihyaluronidase and antielastase, with therapeutic uses in the following diseases: burns, post-burn and postoperative cheloids, eschars, atonic wounds of different etiologies, degenerations of dermic, vascular, tissular and articular collagen such as: skin benign and malignant affections, psoriasis, inflammatory and pruriginous dermatoses, neurodermatitides, radiodermatitides, varicous and post-thrombotic syndrome chronic venous insufficiency, vascular fragility, rheumatic inflammatory conditions and diabetic or endocrine obesity.

10. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids according to claims 1 and 2, conditioned as solution for external use or spray containing 0.5...1% bioactive complex of triterpene acids, as such or in the form of salts, having the following pharmacological effects: trophic and regenerative for dermic, vascular and tissular collagen, antiinflammatory, antioxidizing, membrane fluidity modulator, with therapeutic uses in the following diseases: burns, eschars, atonic wounds of different etiologies, allergical, inflammatory and pruriginous dermatoses, including psoriasis and radiodermatitides.

11. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids according to claims 1 and 2, conditioned as suppositories containing 0.5...1% bioactive complex of triterpene acids, having the following pharmacological effects: antitumorigenic, immunoprotective, antiinflammatory, antioxidizing, trophic and regenerative for vascular, tissular and articular collagen, with therapeutic uses in the following diseases: neoplasm especially in the colon, immunosuppressive conditions, particularly hepatotoxic ones occurred after the treatment with glucocorticosteroids and cytostatics, rheumatic inflammatory affections, hemorrhoidal syndrome.

12. Medicinal product for human and/or veterinary use characterized in that it contains as pharmaceutically active substance the bioactive complex of triterpene acids

described in claims 1 and 2, conditioned as ovules containing 0.5...1% bioactive complex of triterpene, having the following pharmacological effects: antitumorigenic, antiinflammatory, antioxidizing, trophic and regenerative for dermic and tissular collagen, with the following therapeutic uses: vulvo-vaginal trophic disturbances and cervicites, benign or malignant tumour diseases in the vagina and uterine cervix.

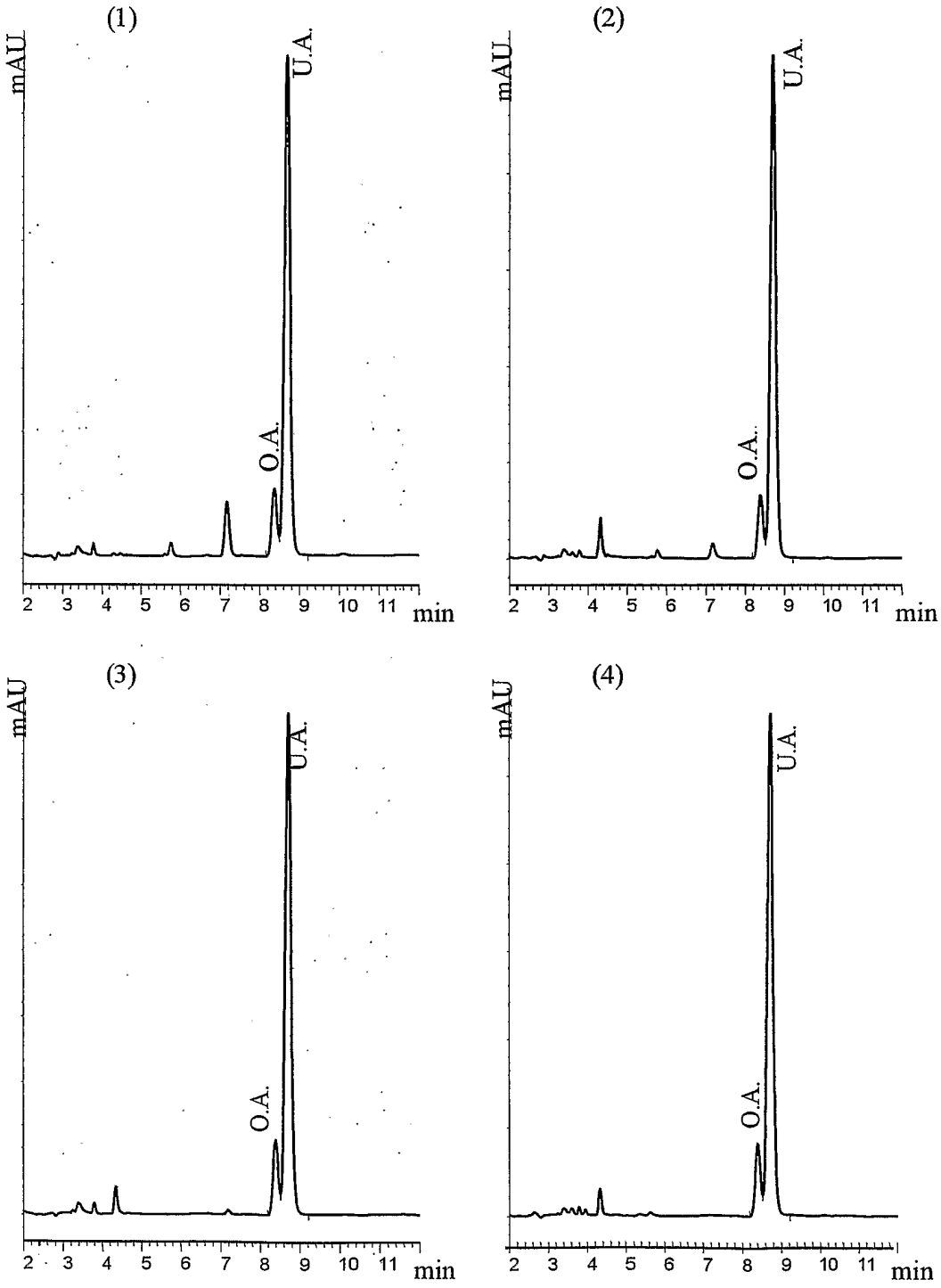


Figure 1. Chromatograms for bioactive complex of triterpene acids produced from: (1) Salvia Sp., (2) Lavandula Sp., (3) Sambucus Nigra, (4) Crataegus Sp.; O.A. = oleanolic acid; U.A. = ursolic acid.