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ISOLATION OF AMYGDALIN FROM SECONDARY PRODUCTS OF MEDICAL OILS PRODUCTION AND INVESTIGATION OF ANTI-CANCER PROPERTIES

Petrosyan H.R.
Institute of Pharmacy, Yerevan State University, Scientific and Production Center "Armbiotechnology" NAS, RA

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The existing technologies consider the main approaches for progressing of complex development of fruit plants raw enriched with biologically and pharmacologically active compounds and food additives. They allow to increase the yield of product's extraction, quality, affectivity of production and lead to the prime cost reduction. Suggested route represents the improvement of phisicochemical methods of product's extraction and refinement as well as the utilization of the secondary compounds formed during main production [7].

In food industry, for the production of galenic and new galenic pharmaceuticals, the problem solution is mainly associated with utilization of complex technologies for the treatment of fruit and herbal raw, providing the wide spectrum of biological effect of BAC (biologically active compounds) obtained in native state, which has a huge importance on the sufficient effect for further uses [1,4,5,11].

Recently there is a growing interest in the natural and harmless food, including the medical oils obtained by the cold pressing of the endemic herbal kernels. They are widely used in applied medicine for prevention of numerous diseases, as well as in food industry as bioadditives [15,16].

During the production, besides the main products (oils, non-saturated fatty acids, carotenoids, flavonoids, etc.) the meal is formed as a secondary waste. The treatment of the latter can often lead to the synthesis of biologically and pharmacologically active valuable compounds, such as cyanogenic glycoside amygdalin or so called vitamin B_{17} [2,3,13]. The growing interest of the pharmacology experts in B_{17} ("Laetrile", "Laetral", "letril") is justified by its anticancer properties and use for local anesthesia [6,9].

It is well known that the content of biologically and pharmacologically active compounds, such as vitamin B_{17}, depends not only on the type of the plant (mostly found in bitter seeds, such as almond, apricot, peach, plum, cherry, etc.), but the geographical location of their growth as well. Vitamin B_{17} is often extracted from the above mentioned bitter kernels by extraction methods [7].

The aim of the research is to develop an effective way of the extraction of a native (the natural structure of the vitamin B_{17} is unchanged) vitamin B_{17} with low prime cost, obtain heptaacetyl derivate of it, to identify their absolute configuration using the modern physicochemical and analytic methods and partially investigate anti-cancer properties on trial models of Sarcoma 180 and on Ehrlich ascites carcinoma.

In this work, the secondary waste of cold pressed medicinal oils of kernels of wild apricot, prune, peach and the bitter almond grown in the ecologically clean areas of Armenia and Artsakh was chosen as a research target for the synthesis of vitamin B_{17}.

Materials and methods

Before the experimental research, an investigation of the areas in the Republic of Armenia and the Republic of Artsakh, where bitter kernels grow, was conducted. Kernels of wild apricot, plum, peach, bitter almond were collected from different regions of Armenia and Artsakh. It should be noted that almond kernels were collected from Meghri region of Armenia, and the kernels of wild apricot, plum and peach were acquired from Artsakh. Initial treatment of kernels (removing wooden skin and drying) was done in the oil production center of SPC "Armbiotechnology" NAN RA. The cold pressed oils were obtained after drying by use of Rawmid Dream modern odm-01 oil presser (Fig. 1).

*** ADDRESS FOR CORRESPONDENCE**

H.R. Petrosyan
YSU, Chair of Pharmtechnology and Pharmacy Economics and Management
Address: *1A Manoogian Street, Yerevan 0025, Armenia*
E-mail: *haykpetrosyan2@gmail.com*
Phone: *(+374) 93 75 31 97, (+374) 43 06 66 73*

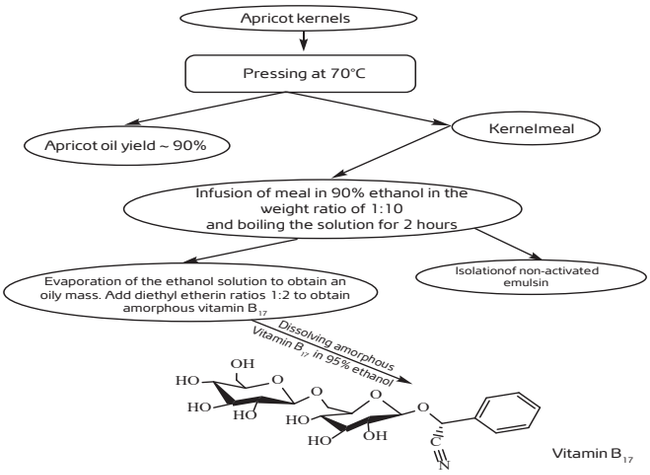

Fig.1. *Technological scheme of complex processing of wild apricot*

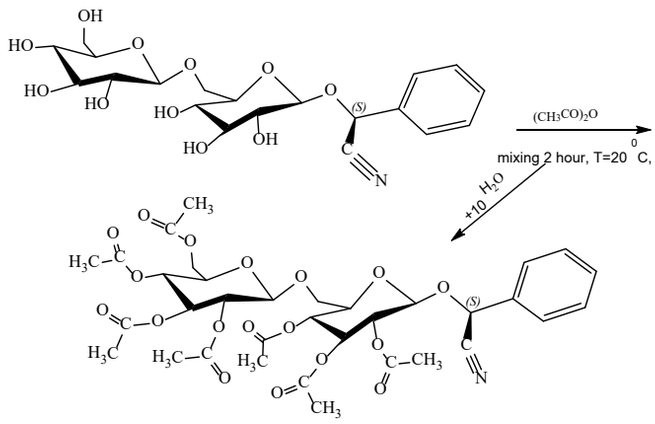

Fig. 2. *Scheme of obtaining Vitamin B_{17} derivate*

Ecologically safe waste generated during the cold pressing of kernels has been extracted by water-ethyl alcohol (90%) system.

For that purpose, waste (80-90g) received from 100 g kernels was added into the triple neck flask, after that 150-200ml ethyl alcohol (90%) was added and the mixture was warmed up at 50°C and 78°C in mixing conditions. Extraction was carried out for 30 min (2 times). Inactivation of emulsion enzyme is going in the first stage of extraction, then isolation of vitamin B_{17} occurs quantitatively [12]. After first stage of extraction and the process of cooling of the extract (up to the ambient temperature) filtration is carried out under residual pressure. The obtained liquid extract in a flat-bottomed flask (200 ml) was moved to a refrigerator (+5°C). The soft mass remaining after filtration was extracted again (30 min) to extract the rest of vitamin B_{17}. The next action was repeated in the same way.

The flasks were stored in a refrigerator for 24 hours. Next day white-yellow sediment (vitamin B_{17}) appeared. The liquid mass was decanted carefully and the flasks were put in an exicator to remove the solvent completely. After the process of drying, amygdalin recrystallized. After recrystallization, the vitamin B_{17} was filtered and then dried at ambient temperature.

In the next stage of the study, heptaacetyl derivative of vitamin B_{17} was obtained (Fig. 2)

For that purpose, 2g (4.4×10^{-3} mol) of vitamin B_{17} was added into a 100ml triple neck flask containing 25ml of acetic anhydride. This substance was mixed during 2 hours at ambient temperature, after which it turned into transparent solution. Then the temperature of solution was decreased to +5°C by ice and salt, after that tenfold volume of distilled water was added and mixed for additional 10-12 minutes. The content of the flask first thickened, then residue emerged shortly after that.

Then the obtained substance was filtrated, the white residue on the filter was washed by distilled water and dried to air-dry condition under hood. Then, the crystals of derivate vitamin B_{17} were recrystallized by 96% ethyl alcohol. After soluting the whole residue, the solution was left in refregerator (8-10°C), where the crystals of heptaacetyl vitamin B_{17} after an hour settled to the bottom as sediment. At first it was filtrated and dried under air conditions, then in vacuum oven (P=40mmHg). Obtained crystals are characterized as the following physical data:

$T_{melt,\ lit.}$ = 173-175°C; $T_{melt,\ sample}$=172-174°C;

$[\ ]_D^{20}{}_{lit.}$ = -34.80° (c=5.0, $CH_3COOC_2H_5$):

$[\ ]_D^{20}{}_{sample}$ = -34.72° (c=5.0, $CH_3COOC_2H_5$).

Results and discussion

It has been found out that the maximum amount of vitamin B_{17} is obtained from the waste of wild apricot oil. For that purpose the deoiled waste is processed by 90% ethyl alcohol at 78°C, required for full inactivation of emulsion in order to avoid breaking of the bond between the aglycon and glycosidic groups in the vitamin molecule, whereas the target product is obtained in its natural state. Then, the obtained vitamin B_{17} was recrystallized by 95% ethyl alcohol. Under the same conditions, 3% (3g), 4.1% (4.1g), 3.2% (3.2g), 2.8% (2.8g) vitamin B_{17}

Table 1

The results of the crystalline of vitamin B_{17} obtained from the secondary waste of bitter kernels

Investigated waste, g	Processing temperature, °C		Yield of vitamin B_{17}, g	
Bitter almond - 100	50	78	0.7	3.2
Prune - 100	50	78	0.7	3
Wild apricot - 100	50	78	1	4.1
Peach - 100	50	78	0.8	2.8

Table 2

Anti-tumor activity of Vitamin B_{17} and its derivate

Compound	Maximum allowable dose	Tumor growth inhibition (%)	
		Sarcoma 180	EAC
Amygdalin	250 mg·kg^{-1}	57.1	0
Heptaacyl derivate	500 mg·kg^{-1}	41.3	0

was obtained from 100g of starting raw materials by extraction of deoiled wastes of almond, wild apricot, black plum and peach kernels, accordingly. The results are presented in the table below.

According to the records in the table, the maximum amount of amygdalin was extracted when the experiments were carried out under the condition of 90% ethyl alcohol boiling point.

Table data clearly show that the maximum amount of amygdalin was extracted from the waste of wild apricot kernels at both 50°C and 78°C. For identification of the obtained vitamin B_{17}, the recrystallized examples were investigated by the modern methods (^{1}H NMR, T_{melt}, andpolarimetric; [ ] D^{20}) of physicochemical analysis. Recrystallized vitamin B_{17} is very soluble in 15 parts of water (20°C), 12 parts of boiling ethyl alcohol and 904 parts of cold ethyl alcohol. It doesn't solve in diethyl ether. Amygdalin is an optically active compound; $[\,]_D^{20}{}_{lit.}$ = -35.51° (c = 5.0, $CH_3COOC_2H_5$): $[\,]_D^{20}{}_{example.}$ = -35.53° (c = 5.0, $CH_3COOC_2H_5$). The melting point is clearly coincided with the literature data; $T_{melt, lit.}$ = 215°C, $T_{melt, example.}$ = 215°C.

Based on the recorded results, an effective way of complex treatment of kernel seeds has been developed. The processes are carried out at atmospheric pressure without use of expensive equipment. At the same time, as a result of use of the secondary raw materials obtained from the primarily treated initial materials, optically pure natural structured vitamin B_{17} with local anesthetic and anti-tumor properties was produced successfully.

In the next stage, the antitumor properties of vitamin B_{17} and its heptaacetyl derivate were studied on experimental models of white mice (20-25 g) with 180 sarcoma and Ehrlich ascites carcinoma at the Toxicology and Chemotherapy Laboratory of the RA Scientific and Technological Center of Organic and Pharmaceutical Chemistry [10]. It is worth noting that the study was conducted in accordance with the guiding principles of animal use and care of the Institutional Committee on Bioethics.

In the primary studies, vitamin B_{17} and its acute heptaacetyl derivate toxicity was considered with simultaneous injections. As a result, the maximum permissible doses of injections necessary for chemotherapy experiments were determined. Vitamin B_{17} and its heptaacetyl derivate was injected to animals for 6 days, using disinfected water for pharmaceutical purposes as solvent.

In case of Sarcoma 180, the antitumor activities of vitamin B_{17} and derivate were evaluated by the tumor growth pressure, and in the case of Ehrlich ascites carcinoma (EAC), by the life expectancy of the injected animals compared to the control group [8,14]. According to acute toxicity studies, the maximum permissible dose of vitamin B_{17} was 500mg·kg^{-1} and 1000mg·kg^{-1} for derivate. For the comparative study of anti-tumor activity, the medicinal dose of vitamin B17 was 250 and 500mg·kg^{-1}, respectively.

The results of antitumor studies are shown in table 2.

According to the data obtained, the extracted vitamin B_{17} shows significant antitumor activity on the Sarcoma 180 model, inhibiting tumor growth by 57.1%. Heptaacetyl derivate is significantly inferior to its anti-cancer properties, compared with vitamin B_{17} inhibiting tumor growth by 41.3%. The samples investigated for Ehrlich ascites carcinoma showed no anti-cancer activity. The data show that there is a need for more in-depth study of the most reliable assessment of the antitumor activity of vitamin B_{17} and its derivatives.

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ԱՄՓՈՓՈՒՄ

ԲԺՇԿԱԿԱՆ ՅՈՒՂԵՐԻ ԱՐՏԱԴՐՈՒԹՅԱՆ ԵՐԿՐՈՐԴԱՅԻՆ ԱՐԳԱՍԻՔՆԵՐԻՑ ԱՄԻԳԴԱԼԻՆԻ ԱՆՋԱՏՈՒՄԸ, ՀԱԿԱՈՒՌՈՒՑՔԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ՀԵՏԱԶՈՏՈՒՄԸ

Պետրոսյան Հ.Ռ.

Ֆարմացիայի ինստիտուտ, Երևանի պետական համալսարան, ՀՀ ԳԱԱ «Հայկենսատեխնոլոգիա» ԳԱԿ ՊՈԱԿ

Բանալի բառեր՝ *դեղաբուսային հումք, վիտամին B_{17}, ամիգդալին, արգասիք-քուսպ, սարկոմա 180, Էռլիխի ասցիտային կարցինոմա:*

Հետազոտվել է դեղաբուսային հումքերի (սև սալոր, հայկական վայրի ծիրան, դեղձ և դառը նուշ) կորիզամիջուկներից սառը մամլմամբ բժշկական յուղերի ստացման երկրորդային արգասիք-քուսպերից նատիվ վիճակում առկա վիտամին B_{17}-ի (ամիգդալին) անջատման արդյունավետ եղանակի մշակման հնարավորությունը: Ցույց է տրվել, որ նատիվ վիճակում առավելագույն քանակի ամիգդալին ստացվում է հայկական վայրի ծիրանի կորիզամիջուկների քուսպի և էթիլ սպիրտի (1:2 հարաբերությամբ) խառնուրդը 78°C-ում մշակելուց և 95%-անոց էթիլ սպիրտով նստեցնելուց հետո: Նման եղանակով հնարավոր է կորզել հումքում առկա վիտամին B_{17}-ի 90%-ը:

Քիմիական մոդիֆիկացայի արդյունքում ստացվել է վիտամին B_{17}-ի հեպտաացիլ նմանակն ու հետազոտվել դրանց հակաուռուցքային ակտիվության մասնակի ուսումնասիրությունը սարկոմա 180 և Էռլիխի ասցիտային կարցինոմա (ԷԱԿ) փորձնական մոդելների վրա:

Ստացված արդյունքները հիմք են տալիս եզրակացնելու, որ հայկական վայրի ծիրանի կորիզամիջուկներից ստացված քուսպը լավագույն ելանյութ կարող է լինել վիտամին B_{17}-ը ստանալու համար:

Պարզվել է, որ վիտամին B_{17}-ի մոդիֆիկացված հեպտաացիլ նմանակը իր հակաուռուցքային ակտիվությամբ զգալիորեն զիջում է ելային վիտամին B_{17}-ին:

РЕЗЮМЕ

ВЫДЕЛЕНИЕ АМИГДАЛИНА ИЗ ВТОРИЧНЫХ ПРОДУКТОВ МЕДИЦИНСКОГО МАСЛА И ИССЛЕДОВАНИЕ ПРОТИВООПУХОЛЕВЫХ СВОЙСТВ

Петросян А.Р.

Институт фармации, Ереванский государственный университет, Научно производственный центр Армбиотехнология, НАН РА

Ключевые слова: *растительное сырье, витамин B_{17}, амигдалин, шрот, саркома 180, асцитная карцинома Эрлиха.*

Исследована возможность разработки эффективного способа выделения нативного витамина B_{17} из вторичного продукта (шрота) производства масел холодного отжима из ядер косточковых плодов лекарственных растений (чернослив, армянский дикий абрикос, персик, горький миндаль). Показано, что максимальный выход нативного витамина B_{17} (амигдалина) получается в результате обработки смеси шрота дикого армянского абрикоса и этилового спирта при 78°C в соотношениях 1:2 с последующим осаждением из 95% - ого этанола. Этим способом можно выделить 90% витамина B_{17}.

В результате химической модификации было получено производное витамина B_{17}, а противораковые свойства были частично исследованы на экспериментальных моделях саркомы 180 и асцитной карциномы Эрлиха.

На основании полученных данных можно утверждать, что шрот дикого армянского абрикоса является наилучшим исходным сырьем для получения витамина $B_{17.}$ Было обнаружено, что витамин B_{17} проявляет противоопухолевую активность, подавляя увеличение саркомы 180 на 57%. На модели асцитной карциномы Эрлиха противоопухолевой активности не обнаружено. Было установлено, что модифицированное производное витамина B_{17} по противораковым свойствам значительно уступает витамину B_{17}.