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ABOUT RESTORATION OF THE CENTERS ANTHROPOGENIC-DEGRADED SANDY SOILS OF DESERT PASTURES OF THE SOUTHEAST OF KAZAKHSTAN

**Abstract**

In article are given the factors promoting formation of desert sandy soils in South Lake Balkhash region where they at excess anthropogenic loading easily losing structure is formed turn into the developed sands, forming the centers of mobile barkhans.

Results of definitions of a thermal condition of sandy barkhans have shown increase of temperature of the sand from March to August months with the subsequent decrease in September. Studying of the water regime was shown about its low supply, especially in summer months, and also in an early autumn and is the main limiting factor of survival of saplings.

The low probability of survival of saplings of sand binding brush wood because of low field humidity of a root layer (1-3%) in the conditions of high temperature (25ºC) and low relative humidity (29%) of a ground layer of air in summer months is established.

***Key words:*** pasture, moisture, desert, barkhan, *Calligonum*, saplings.

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MODERN METHODS TO DERIVE MEDICINES FROM CANNABIS

**Abstract**

The paper presents methods to derive medicines from wild cannabis from the Chuya valley. Medicines made of substances extracted from cannabis are used to treat HIV-infected patients, leukemia, epilepsy, asthma, glaucoma, disseminated sclerosis, ulcers, and other neuromuscular disorders. The paper also provides methods to derive the chemically pure tetrahydrocannabinol from wild cannabis. The study was conducted in association with German-based THC Pharm GmdH, the only company in Europe having the sale and manufacture license for a cannabis-based medicine - dronabinol.

***Keywords:*** tetrahydrocannabinol, cannabidiol, Levonantradol, Marinol, DNA, RNA, Cannabis sativa, Cannabis ruderalis, Cannabis sativa L., indica, chromatography, chlorophyll, extract, cannabinol, cannabichromene.

One of the important and hotly debated problems in the world pharmacy is the rationality of cannabis legalization for medical purposes. These substances have been already widely used in hospitals of Canada and the United States of America.

*Cannabis* is one of the superpotent medicinal plants and was used in ancient times for medicinal purposes. In Asia, the plant in the form of a variety of medicines was recommended for the treatment of a number of diseases such as cough, lassitude, rheumatism, asthma, delirium tremens, headache, nasty forms of menstruation, etc., although its consumption fell due to the

introduction of synthetic sleep-inducing drugs and analgesics. For a long time the plant was included in the Pharmacopoeia of many world countries, but then it was prohibited. The discovery of a method reducing the intraocular pressure with the help of cannabinoids revived interest in their use for therapeutic purposes, in particular for glaucoma treatment. Marinol is used for the prevention of sickliness and vomiting associated with cancer chemotherapy. It is able to eliminate sickliness and vomiting in doses that do not affect thinking. Over the past few years, the world community is actively working to derive new medicines from cannabis. Countries such as Belgium, France, Germany, Netherlands, United Kingdom and Finland conduct experiments to determine the effectiveness of marihuana for the treatment of the following disorders:

- ✓ sickliness and vomiting during chemotherapy and radiotherapy;
- ✓ glaucoma;
- ✓ disseminated sclerosis;
- ✓ exhaustion and loss of appetite caused by AIDS;
- ✓ spinal injuries or diseases;
- ✓ chronic pain such as arthritis;
- ✓ Tourette's syndrome

Cannabinoids are biologically active substances of special structure that naturally occur exclusively in plants of the hemp family (*Cannabaceae*). Plant cannabinoids are also called phytocannabinoids. Cannabis contains more than 421 chemical substances, including 61 cannabinoids. The precursor of all plant cannabinoids is cannabigerolic acid, which is transformed into cannabichromenic, cannabidiolic and delta-9-tetrahydrocannabinol acids under the influence of three independent cyclase enzymes. Due to decarboxylation these acids give free cannabinoids - cannabichromene, cannabidiol and delta-9-tetrahydrocannabinol, respectively. The rest of cannabinoids are products of biotransformation of main cannabinoids.

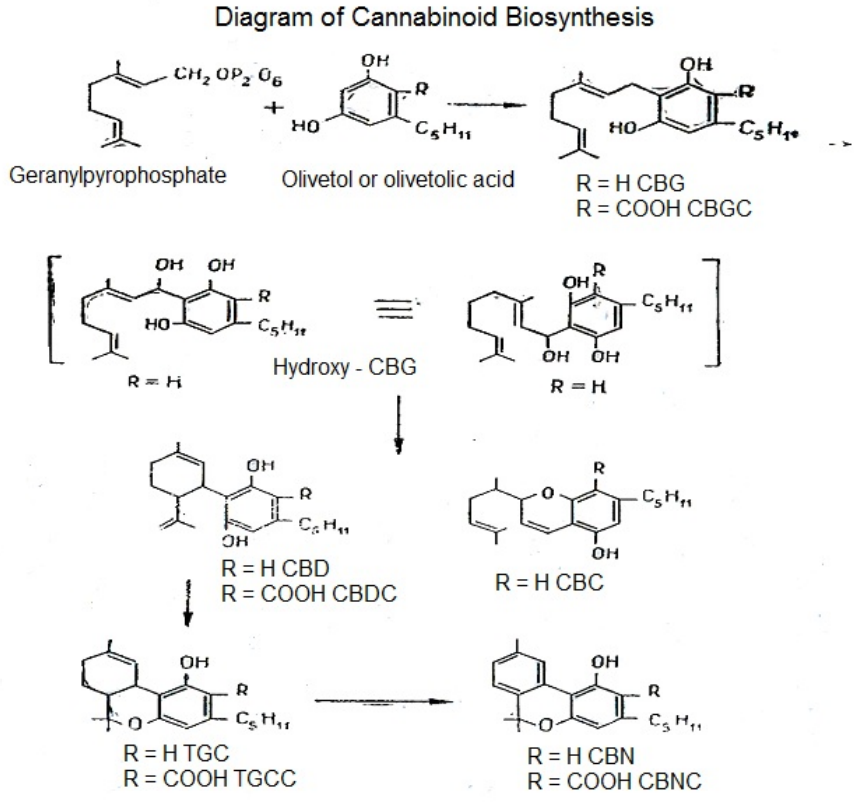


Figure 1. Cannabinoids Biosynthesis.

Modern literature has a lot of information about experiments to test effects of some synthetic and natural components of marihuana for the treatment of certain diseases. Currently, cannabis is very popular among modern drug manufacturers since seriously ill patients usually have no addiction to cannabis unlike opiates. Diluted cannabinoids are modified quickly [1-5]. Only tetrahydrocannabinol (THC) is known for more than 60 isomers. Having stabilized the structure of this compound one can easily obtain a powerful remedy for various diseases that was done by ROXANE (since 1999 Unimed. Solvay Pharmaceuticals, Inc, USA), an American company that in 1985 put on the market a medicine called Marinol. Attitude to cannabis derivatives is now becoming positive. Recently, it was proven that THC does not accumulate in fat cells of the human brain, does not kill them, and does not make it lazy and dull. THC's major target is the brain, where it interacts with specific receptors $CR_1$, $CR_2$ (18, 20, 21), which maximum number is located in the brain cortex (anterior), limbic system, hippocampus and cerebellum [6]. Moreover, Alan Leshner [6] has already selected a component (called SR141716) that blocks cannabinoid receptors in the brain and thereby their influence. Cannabinoids-like compounds have been already proved to be usual components of a human body. Their role in the body is still unknown. Although the identified component SR141716 inhibits chemical processes in the brain and may be used to treat rare cases of THC-addiction.

When people say about the use of cannabis, they imply the use of inflorescence leaves and apical leaves. Fur filled with cannabinoids is formed on their inner surface. Usually, dry leaves of wild and cultivated narcotic plants accumulate 0.03 to 4.8 of psychoactive ingredient - delta-9-tetrahydrocannabinol or THC.

Modern cultural narcotic varieties contain up to 20% of THC (dry weight) in their tops. Other cannabinoids such as cannabidiol (CBD), cannabinol (CBN) and cannabichromene contained in the plant do not have a psychoactive effect, but are biologically active, in particular they inhibit the synthesis of DNA, RNA and proteins.

The term 'medical marihuana' covers several substances that have different legal and medical status:

1. Cannabis plant and its resin - any part of Cannabis sativa L., indica, ruderalis, or a resin derived from its tops.
2. Extract of cannabis - usually oil derived from the plant and medicines derived from the oil; known as hash oil.
3. Cannabinoids. This is the class of chemical substances that have a general structure. Cannabis contains more than 60 cannabinoids.

The term 'Botanical Drug Substances' derived from a cannabis plant material is officially used in the USA instead of the term 'cannabis extract'. Cannabis plants include a wild genotype of *Cannabis sativa* and its varieties and other genotypes of natural origin, containing different amounts of individual cannabinoids. According to the Guidance for industry Botanical Drug Products Draft Guidance, August 2000, US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research, it is defined as 'a drug substance derived from one or more plants, algae, or macroscopic fungi, and prepared from botanical raw materials by one or more of the following processes: pulverization, decoction, expression, aqueous extraction, ethanolic extraction, or other similar process'. The term 'Botanical Drug Substances' does not cover chemically pure or modified substances derived from natural raw materials. This means that in the case with cannabis the term 'Botanical Drug Substances' does not include chemically pure cannabinoids.

Cannabis Based Medicinal Extracts (CBMEs) prepared through one of the above methods are classified as botanical drug substances in accordance with the definition given in the Guidance for industry Botanical Drug Products Draft Guidance, August 2000, US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research.

Botanical drug substances derived from cannabis plants include the primary extract obtained through the process such as maceration, percolation, extraction with solvents such as Cl to C5 alcohols (i.e. ethanol), Norflurane (HFA134a), HFA 227 and liquid carbon dioxide at low temperatures. The primary extract may be further purified through the batch extraction, distillation or chromatography. Further conversion of botanical drug substances into botanical drug products is defined in the Guidance for industry Botanical Drug Products Draft Guidance, August 2000, US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research as follows: a botanical product that is intended for use as a drug or a medicine prepared from a botanical drug substance.

The Chuya valley in Kazakhstan with its huge reserves of wild cannabis, the lack of domestic superpotent drugs gave rise to the need to conduct the study to derive a Kazakhstani pharmaceutical drug. This forms the objective of this study.

*Development of a technology to derive the highly purified cannabinoids from low- and high-cannabinoid cannabis.*

Currently, there are two cannabis drugs produced officially in the world - Marinol and Dronabinol. The first one is manufactured in the USA by the sequential chemical synthesis of Δ-9-THC starting from two carbon molecules. Dronabinol is manufactured by German-based THC Pharm GmbH by the synthesis from non-narcotic cannabinoids. In both cases, the use of cannabis as a raw material with a high content of THC is illegal in these countries.

The literature contains information about how to derive the delta-9-THC from marihuana [7]. In this vein, Wolver et al (1942) published a paper that describes the THC extraction by preparing an ethanolic extract of red color, through its purification with ester by repeated distillation in vacuum and the column chromatography with silica gel and by passing it through activated alumina and TCA. The product obtained was not chemically pure.

In 1960, De Ropp [8] separated THC by using tops of *Cannabis sativa's* leaves. After extraction with methanol, he chromatographed the extract through a column of Celite followed by vacuum distillation. Extract purity was controlled through chromatography of extracts on paper. Most pure cannabinoids were derived from hashish by Gaoni and Mechoulam [9] in 1964. THC was obtained by repeated chromatography through a column of florisil and alumina, subsequent synthesis of intermediate compound of 3,5-dinitrophenylurethan with THC, purification of this compound and mild hydrolysis. Its purity was controlled through chromatography on plates with silufol and spectroscopic analysis (IR and NMR).

In 1965, Korte et al [10] derived a rough extract from female plants - Cannabis sativa indica and Cannabis sativa non indica. In order to remove impurities such as chlorophyll, carotenoids, xanthophylls, the extract was chromatographed through a column of activated alumina. All cannabinoid fractions were concentrated as hashish oil of red color. Further purification was carried out based on Gaoni and Mechoulam's scheme (1964).

In 1967, Gaoni and Mechoulam reported again the THC purification. They used the following scheme: cannabinoids were extracted from hashish with hexane. The extract was separated into acidic and neutral fractions. The acidic fraction was chromatographed through a column of frosil or alumina oxide washed by acid. It was eluted with a mixture of pentane and ether with the gradually increasing polarity of the solution. THC was in the fraction containing 15% of ether in pentane. Further chromatography was associated with the generation of a crystalline derivative - THC-3,5-dinitrophenylurethane and its subsequent hydrolysis.

In 1972, Verwey Witte published a method for the isolation of THC-A. Cannabinoids were isolated with hexane from hashish, the extract was then placed in a vessel with two layers - 2% NaOH and 2% sodium sulfite. The alkaline layer was acidified with $H_2SO_4$ pH≤2 that results in the acid cannabinoids settling. THC was derived by evaporation of the ether solution THC-A at $300^0$C.

Whittle Brian [11] had to summarize all of these methods and patent with the U.S. Patent Office. A positive opinion was obtained in 2003. He called THC, CBD, CBN, THCAc, CBDCOOH, THCV as medicaments being so both independently and in combination, both natural (from plants) and synthetic, and their receptors. Unlike previously used methods, a liquid carbon dioxide was used for the extraction.

We also developed some preparative methods to derive the chemically pure THC from wild cannabis. The study was conducted in association with German-based THC Pharm GmdH, the only company in Europe having the sale and manufacture license for a cannabis-based medicine - dronabinol. We have developed a detailed method to derive the narcotic substance THC from wild cannabis. Since this section has a number of know-how, some stages are not described in detail.

**A brief description of the process of deriving cannabinoids from Chuya cannabis**

1. Plants are cut down in the period from July to September. Gross productivity of continuous wilds of cannabis is usually around 10-35 centner/ha, cultural crops - about 30-60 centner/ha. Leaves and buds are separated from stems by simple shaking after drying. They are thoroughly mixed to a homogeneous mass, and the dry mass is placed in bags weighing approximately 10-15kg.

2. The bags of dry leaves and buds are transported under police guard to a laboratory and placed in a special storage room.

3. Total loss of raw materials after drying is about 80%. About 15% of the total weight of the plants accounts for leaves and buds. About 20% of the total weight of leaves remain on branches. Thus, 1,000 kg of freshly cut cannabis give about 200 kg of dry plants. 30kg out of 200kg accounts for leaves and buds. After separating stems from leaves, we obtain about 24kg of dry leaves and buds, where stones, sand and other debris make up about 2kg.

4. Usually the Chuya cannabis contains 2 to 8% of THC. Dry leaves and buds (5kg) are placed in 30L stainless steel drums, which are filled with petroleum ether, capped and shaken from time to time. In 24 hours, the extract is poured into other drum containing another 5 kg of leaves, and the process repeats. There are usually 5 drums. After the extract passes through five drums, it is saturated and then evaporated in a rotary evaporator in vacuum at $60^0$C. The remaining residues of plant materials in drums are again filled with petroleum ether, and the process repeats. Thus, cannabinoids are repeatedly extracted from plant materials with petroleum ether containing no aromatic impurities.

5. The extract obtained is evaporated under vacuum with heating. After the extraction, cannabinoids are purified by liquid chromatography using chromatographic columns of different diameter. Various factions of cannabinoids purified by this way are esterified with diazomethane. Then, they are purified by distillation under high vacuum. As the result, we obtain the following cannabinoids:

1. Tetrahydrocannabinol
2. Kannabinodiol
3. Metilkannabinolat
4. Metiltetrahydrocannabinolat

6. Interest output of pure raw materials is about 45% of the initial content of THC. Thus, one ton of pure raw cannabis contains 30 kg of THC immediately after drying. This gives 5-8kg of pure cannabinoids.

We jointly with THC Pharm GmbH started developing a method to derive THC from cannabidiol (CBD). The need to develop the method is associated with the availability of cannabis with a high content of the compound in the Chuya valley; more simple CBD purification procedure in comparison with THC by the re-settling of CBD; the availability of CBD in the non-narcotic cannabis cultivars.

Samples of cultivated non-narcotic cannabis were taken. Their cannabidiol content was 3%. The samples were dried and extracted as described above. CBD was settled out by acidifying the solution, re-dissolved and purified several times by distillation. CBD was isomerized into $\Delta^1$-THC. This process was carried out in the presence of a catalyst - boron salts in the methylene chloride solution. In 30 minutes, equal amount of water and ether was added. The organic layer was separated and washed with sodium bicarbonate and sodium sulfate solutions. After evaporation and chromatography through a column of Florisil, it was eluted with pentane with the increasing concentration of ether. The first component of $\Delta^1$-THC was eluted at an ether concentration of 1%, the last $\Delta^9$-THC – at a 2-4% ether in pentane. Pure THC was obtained after repeated distillation. This method is cheaper than the previously developed one since the separation of THC from other cannabinoids is a very labor-intensive and expensive process. This method is much easier and faster. The THC content is 99.5%. The quality of THC is not different from THC sold through a network of pharmacies by THC Pharm GmbH in Germany.

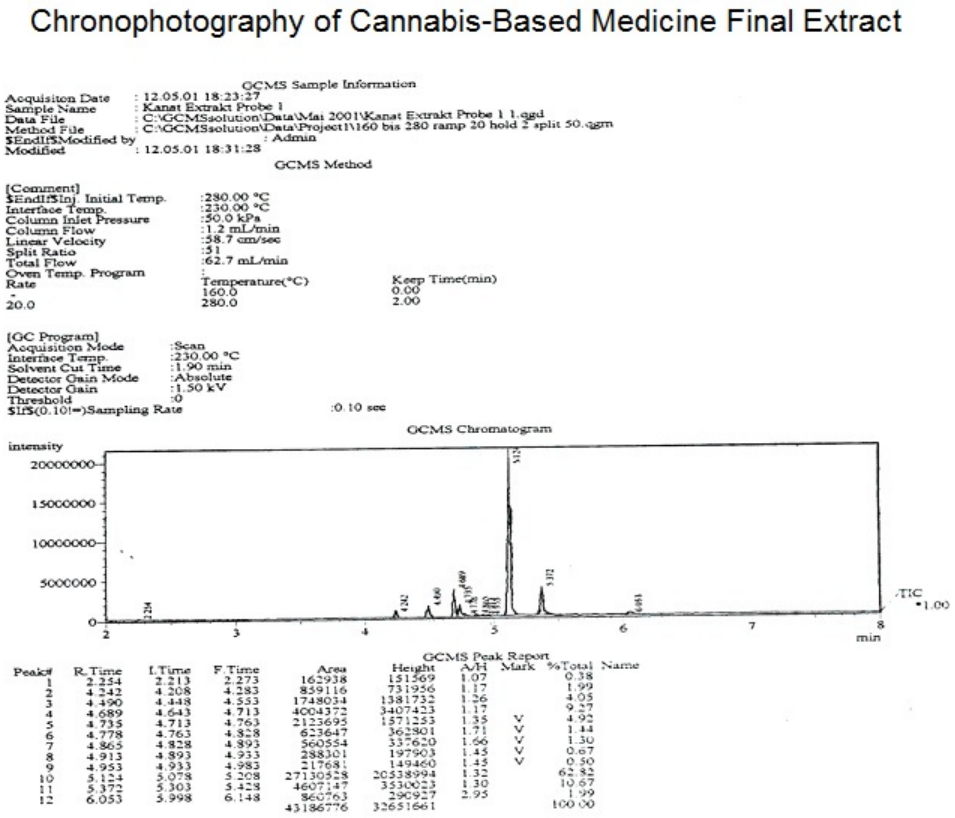


Figure 2. Chromatography of Cannabis-Based Medicine Phenolic Extract

Over the years, all the work focused on the development of a low-cost method to derive cannabinoids. The previously developed method was very expensive as reagents for the extraction and chromatography are not produced in Kazakhstan. In addition, a large number of publications have proven that pure THC is less therapeutically active in contrast with THC in combination with other cannabinoids. For example, pure THC kills the pain to a lesser extent than in the presence of small amounts of an extract from inflorescences. The pharmacological explanation of this phenomenon is currently under investigation. However, completely different

results are obtained in some cases of simultaneous administration of THC and cannabidiol (CBD) in preclinical trials. For example, some results indicate the modification of the psychoactive effect of cannabinoids that enables to understand the therapeutic effect of rough extracts from different parts of the world. The increasing effect is observed in case of joint administration of THC and CBD, although the pharmacological effect of both components is very different. The THC toxicity is clinically proven to be higher for the organism than a mixture of THC and CBD. Tetrahydrocannabinovarin, a THC analogue, has stronger therapeutic effect than THC and CBD. The study of this compound has only just begun, but its curative effect has been already patented by Brian White [11]. Since the Chuya valley has an enormous number of varieties with different combinations of cannabinoids, it is not difficult to find right plants and develop a medicine with the right combination of cannabinoids.

The methods are based on:

1. Screening method
2. Batch extraction
3. Cold extraction followed by chromatography

Based on the first method, leaves were cut down from tops of branches and stems before the first frost. They were then dried and screened with the THC concentration of about 20%. After receiving special equipment from Pollinator, the screening was mechanized and became more efficient.

Based on the second method, the extraction was conducted using petroleum ether, the extract was then sublimated and dried. The THC concentration was about 62%. It included also CBD and CBN – non-narcotic cannabinoids.

Physical properties of fur on leaves were applied for the third method: at $0^0$t it comes off the leaf and rolls into tiny balls. The fur was passed through a screen having a mesh diameter of 100 and 40 microns. As the result, the extract contained 60% of THC, 9% of CBD, 10% of CBN, and 5% of CBDCOOH.

The most expensive Dutch hashish Ice-O-Lator obtained using this analogous method contains only 15-20% of THC. This is due to the high quality of plant ‘dust’ that we collected (without screening) in the Chuya valley in late September. Using these simple methods, we derived three medicines. Their diluted solutions were transferred for preclinical trials. According to the preliminary estimate, they effectively influence on animals and model systems.

Thus, during the study of quantitative and qualitative composition of cannabinoids from the Chuya valley, we have developed 3 schemes of deriving pharmaceutical medicines from cannabis:

1. A 99% THC medicine. It was derived by the repeated settling and chromatography of an extract from cannabis inflorescences through a column of silica gel.

2. A 62% THC medicine. Its composition also includes CBD and CBN – non-narcotic cannabinoids. The combination of these three cannabinoids gives the synergistic effect. The medicine is closer to the natural extract and derived by the repeated settling of the extract from cannabis inflorescences. Its production scheme is much easier and cheaper than the first one.

3. A 60% THC medicine. The medicine is derived by the settling of the fur containing cannabinoids at low temperature and by its subsequent filtering through a system of screens with different mesh sizes. This scheme is the simplest and cheapest.

Work with the medicines obtained is possible for research purposes only. Market promotion of our medicines requires a lot of permits that is in contradiction with the Criminal Procedure Code of the Republic of Kazakhstan. The industrial processing of cannabis in order to sell a cheap medicine on the market requires permission from the Government and a number of legislative measures.

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## СОВРЕМЕННЫЕ МЕТОДЫ ПОЛУЧЕНИЯ МЕДИЦИНСКИХ ПРЕПАРАТОВ ИЗ КОНОПЛИ

**Аннотация**

В статье рассматривается методы получения медпрепаратов из дикорастущей шуйской конопли. Лекарства, созданные на основе веществ, выделенных из конопли, используют при лечении ВИЧ-инфицированных больных, лейкемии, эпилепсии, астмы, глаукомы, множественных склерозов, язв, и других нервно-мышечных расстройств, а также методы получения химически чистого тетрагидроканнабинола из дикорастущей конопли.

***Ключевые слова:*** тетрагидроканнабинол, каннабидиол, Маринол, ДНК, РНК, Cannabis sativa, Cannabis ruderalis, Cannabis sativa L., indica, хромотография, хлорофилл.