EXTRACTS OF ZANTHOXYLUM BUNGEANUM, PHARMACEUTICAL AND COSMETIC FORMULATIONS CONTAINING THEM

The present invention relates to a novel extract obtained by extraction of the pericarp of Zanthoxylum bungeanum with carbon dioxide and to the pharmaceutical and cosmetic formulations containing it. This extract has antiinflammatory and analgesic activities and can be used for the treatment of itching.

Pericarp of Zanthoxylum bungeanum is commonly used in China as spices for seasoning food. Moreover, in Chinese and Indian folk medicine, this part of the plant is used as local anaesthetic and for the treatment of dysentery. The essential oil, which contains a series of monoterpenes such as 1,8-cimol, linalool, 4-terpinol, caryophyllene, limonene and the like, is reported as a strong repellent against insects.

Recently, EP 568001 disclosed the antiviral activity of the skin of this fruit.

In US 5,137,912 chelcrythrine, extracted from the root bark of Zanthoxylum simulans (synonym of bungeanum), was disclosed to be active in the prevention of thrombosis. According to JP 01294657, the extraction of the pericarp with organic solvents provides an extract containing isobutylamides exerting local anaesthetic effect already 30 seconds after the application on the tongue and lasting up to 20-80 minutes.

It has now surprisingly been found that the extract of the pericarp of Zanthoxylum bungeanum, prepared by extraction with carbon dioxide in supercritical conditions, has a remarkable analgesic activity without

exerting the local anaesthetic activity characteristic of the extracts obtained by means of solvents. The product of the present invention is prepared extracting the pericarp of Zanthoxylum bungeanum, finely ground or transformed into pellets, with carbon dioxide under pressure conditions ranging from 150 to 300 bars, preferably under 180-230 bars, at temperatures ranging from 35 to 55°C, preferably at 35-40°C.

The resulting extract can either be used as it is, after removing the extraction water, or further be purified by partitioning it with immiscible solvents such as aliphatic alcohols and aliphatic hydrocarbons, preferably n-hexane or petroleum ether.

The resulting extracts proved to exert a marked analgesic activity when applied percutanously to humans; therefore they are valuable for use both in the pharmaceutical and cosmetic fields.

In the pharmaceutical field, they are useful for alleviating pain in diabetic microangiopathy, in hemorrhoidal pain, in burns and in any form of local pain. A further application of the extracts of the invention is the treatment of itching.

In the cosmetic field, the extracts according to the invention are useful in depilatory creams, after-sun formulations, shaving lotions and creams, and in all the skin treatments requiring local analgesic and anti-itching actions.

The anti-itching activity of the extracts of the invention was evaluated through electrophysiological measurements using the experimental model of the rat ischiatic nerve-musculus extensor digitorum longus (EDL) preparation ***in vitro***.

Adult Sprague-Dawley rats killed by an intracardial Penthotal overdose were used. The EDL muscle with a long tract of ischiatic nerve was dissected and placed in a container for electrophysiology with a Ringer solution which was substituted every 10 minutes. The tested extracts, preferably added with a surfactant, were dispersed in different concentrations, thereby coming into contact with nerve fibers, neuromuscular junctions and muscle. For the electrophysiological tests, an intracellular microelectrode filled with 3M KCl was placed in muscle cells for recording the electrical phenomena (miniature endplate potentials, endplate potentials, membrane potentials of the muscle cell) both spontaneous and induced by stimulation of the nerve. For this purpose, the nerve was linked to a stimulator and optionally stimulated once a second. The electric events in the muscle cells were detected by the microelectrode linked to a signal amplifier and made visible through a digital oscilloscope.

The extracts of the invention, when tested at concentrations ranging from 0.0005 to 0.002%, exert a strong, transitory activating action on the neuromuscolar synaptic transmission, evidenced by the increase in the frequency of the miniature endplate potentials and by the appearance of spontaneous endplate potentials. Conversely, the extracts obtained with solvents, having local anaesthetic activity, reduce and inhibit the transmission of the nervous impulse.

The analgesic activity of the tested extracts was evaluated in 10 healthy volunteers through an evaluation study or the skin thermal sensitivity.

A progressively heated thermal probe was placed on the scapular area of the subject 30 minutes after treatment with the tested product or with the corresponding placebo. The value of the temperature considered painful by the subject was measured. 0.5 ml of an emulsion prepared according to Example VII, containing 0.5% of an extract of Zanthoxylum bungeanum prepared according to Example I, were administered. The results reported in the following show the analgesic activity of the tested extract.

Treatment	T_0	T_{30min}
Placebo	45.7±1.0	45.3±0.4
Extract	46.0±0.8	47.2±0.4

The extracts of the invention can be formulated in the form of creams, lotions, foams, gels, for the administration on the skin or mucosas, soft-gelatin capsules, hard-gelatin capsules, tablets or suppositories; preferably the extract of the invention is formulated in creams or foams for the cutaneous treatment or in soft-gelatin capsules, chewable tablets or suppositories for the systemic route.

The extract dosages in the formulations range between 5 and 100 mg per dose in the formulations for the systemic use, whereas they vary from 0.05 to 1% in the topical formulations.

The examples reported hereinbelow further illustrate the invention without limiting it.

EXAMPLE I - Preparation of a Zanthoxylum bungeanum lipophilic extract

10 Kg of pericarp of Zanthoxylum bungeanum are extracted according to the procedure reported below, in a 25 L extraction plant for supercritical gas, equipped

with two separators as condensers for fractioning the extract.

10 Kg of pericarp mechanically dried after harvesting at a temperature not higher than 60°C, were extruded into cubes and extracted with CO_2 in supercritical conditions under the following experimental conditions:

- temperature: 35°C in the extractor, 30°C in the first separator and 20°C in the second separator;
- pressure: 180 bars in the extractor, 100 bars in the first separator and 50 bars in the second separator.

The CO_2 flow was 10 L per minute for 45 minutes. The extract was concentrated in the second separator, whereas most water present in the vegetable matrix was concentrated in the first separator . After drying the extract in the second separator under vacuum at a temperature not above 40°C, 1.5 Kg of pasty extract were obtained, which was slightly coloured in yellow, intensely scented and had a content in isobutylamides of about 25% by weight. HPLC analysis was carried out on a Hibar RT LiChrospher 100RP-18 column with the elution profile (1mL/min) reported in the table. The injected amount was 5 μL of a solution of concentration 2 mg/mL. The chromatogram is reported in the figure.

TABLE

TIME (min)	WATER (%)	ACETONITRILE (%)	NUMBER OF THE GRADIENT CURVE
0	60	40	-
20	60	40	6
30	10	90	6
35	60	40	6
40	60	40	6

EXAMPLE II - Preparation of a Zanthoxylum bungeanum lipophilic extract

10 Kg of pericarp mechanically dried after harvesting at a temperature not higher than 60°C, were extruded into cubes and extracted with CO_2 in supercritical conditions under the following experimental conditions:

- temperature: 40°C in the extractor, 30°C in the first separator and 20°C in the second separator;
- pressure: 205 bars in the extractor, 100 bars in the first separator and 50 bars in the second separator.

The CO_2 flow was 10 L per minute for 45 minutes. The extract was concentrated in the second separator, whereas most water present in the vegetable matrix was concentrated in the first separator. After drying the extract in the second separator under vacuum at a temperature not above 40°C, 1.5 Kg of pasty extract were obtained, which was slightly coloured in yellow/green and had the same chemical-physical characteristics as the extract of example I, and an about 20% content in isobutylamides.

EXAMPLE III - Preparation of a Zanthoxylum bungeanum lipophilic extract

10 Kg of pericarp mechanically dried after harvesting at a temperature not higher than 60°C, were extruded into cubes and extracted with CO_2 in supercritical conditions under the following experimental conditions: temperature in the extractor 40°C and pressure 230 bars. The ratio of CO_2 used for the extraction of the drug to be extracted was 27-45 Kg per Kg of drug. The extract was concentrated in a separator under 50 bars at 20°C. After drying under

vacuum at a temperature not higher than 40°C, 1.3 Kg of pasty extract were obtained, which was slightly coloured in yellow/green, and had the same chemical-physical characteristics as the extract of example I.

EXAMPLE IV - Preparation of a purified lipophilic extract of Zanthoxylum bungeanum

0.5 Kg of lipophilic extract of pericarp of Zanthoxylum bungeanum prepared according to Example I were dissolved in 2.5 L of 95% aqueous methanol and extracted three times with 0.5 L each of n-hexane. The hexane phase was counterwashed with methanol using as marker the isobutylamides which should remain in the methanol phase. The inactive hexane phase was removed, whereas the methanol ones were combined, diluted with 0.6 L of water and re-extracted twice with 0.6 L of n-hexane. The combined hexane phases were decolourized with 0.3% charcoal, dried over Na_2SO_4 and concentrated to an oil at a temperature not higher than 40°C under vacuum, to obtain 0.22 Kg of an oily extract with honey-like consistence, having a content in isobutylamides of about 50%.

EXAMPLE V - Zanthoxylum bungeanum alcoholic solution

100 ml contain:

Zanthoxylum Bungeanum 20% solution in Oleyl Alcohol	0.50 g
Cyclomethicone	20.00 g
Alcohol	100.0 ml

EXAMPLE VI - Zanthoxylum bungeanum analcoholic solution

100 ml contain:

Zanthoxylum Bungeanum 20% solution in Oleyl Alcohol	0.50 g
PPG-26 Buteth 26 and	

PEG-40 Hydrogenated Castor Oil		5.00 g
Methyl Chloroisothiazolinone and Methyl Isothiazolinone		0.10 g
Purified water	q.s. to	100.0 ml

EXAMPLE VII - Zanthoxylum bungeanum emulsion

100 g contain:

Zanthoxylum Bungeanum 20% solution in Oleyl Alcohol	0.50 g
Isohexadecane	5.00 g
Glycerin	4.00 g
C_{12-15} Alkyl Benzoate	2.00 g
Cyclomethicone	2.00 g
PEG-20 Glyceryl Stearate and Glyceryl Stearate	2.00 g
Lanolin wax	1.00 g
Acrylates/C_{10-30} Alkyl Acrylate Crosspolymer	0.50 g
Cetyl Alcohol	0.50 g
Imidazolidinyl urea	0.30 g
Phenoxyethanol	0.50 g
Methyl paraben	0.20 g
Tocopherol	0.10 g
Disodium EDTA	0.10 g
Water	q.s. to 100.0 g

EXAMPLE VIII - Zanthoxylum bungeanum fluid emulsion

100 g contain:

Zanthoxylum Bungeanum 20% solution in Oleyl Alcohol	0.50 g
PEG-20 Glyceryl Stearate and Glyceryl Stearate	10.00 g
C_{10-18} Triglycerides	10.00 g
Glycerin	5.00 g

Wheat Germ Oil		2.00 g
Dimethicone		2.00 g
PPG-25 Laureth-25		2.00 g
Cetyl Alcohol		1.00 g
Hydroxylated Lanolin		0.50 g
Imidazolidinyl urea		0.30 g
Hectorite (e) Hydroxyethyl		0.50 g
Phenoxyethanol and Methylparaben and Ethylparaben and Propylparaben and Butylparaben		0.50 g
Tocopherol		0.10 g
Water	q.s. to	100.0 g

CLAIMS

1. Extracts of Zanthoxylum bungeanum obtainable by extraction of the pericarp with supercritical CO_2.

2. Extracts as claimed in claim 1 obtainable by extraction with CO_2 at temperatures ranging from 35 to 55°C and under pressures ranging from 150 to 300 bars.

3. Extracts as claimed in claims 1 and 2 having a content in isobutylamides ranging from 20% to 50% by weight.

4. Pharmaceutical compositions containing as active ingredient an extract of claims 1 to 3 in admixture with suitable excipients.

5. Cosmetic compositions containing as active ingredient an extract of claims 1 to 3 in admixture with suitable excipients.

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