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TANACETUM PARTHENIUM EXTRACT

The present invention relates to a Tanacetum parthenium extract substantially free from α-unsaturated γ-lactons.

Furthermore, the invention relates to a process for the preparation of the extract and to pharmaceutical and cosmetic compositions containing the Tanacetum parthenium extract substantially free from parthenolide.

TECHNOLOGICAL BACKGROUND

The extracts of Tanacetum parthenium, a plant belonging to the family of Asteracee/Composite, also known under the names Altamisa, Crisanthemum, Leucanthemum, Pyrethrum parthenium or under the common name "feverfew", have traditionally been used in the treatment of migraine, vertigo, arthritis, menstrual disorders, fever, toothache, stomach ache and insect bites. The extracts of Tanacetum parthenium, in addition to volatile oils of various nature (mono- and sesquiterpene components), flavonoids, tannins, pyrethrin, also contain terpenoids of the family of sesquiterpene lactones known under the names germacranolides, guaianolides and eudesmanolides. Said compounds are characterized by an α-unsaturated γ-lactone structure and comprise in particular the compounds known under the names parthenolide, 3-ß-hydroxy-parthenoide, costunolide, 3-ß-hydroxy-costunolide, artemorin, 8-α-hydroxy-estafiatin and chrysanthemonin.

The presence of said sesquiterpene lactones is considered the basic condition for the pharmacological activity of the extracts (J. Pharm. Pharmacol. 1992, 44:391-395).

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The attention has been focused, in particular, on parthenolide, which is thought to be the fundamental active ingredient of the extract, but also the main responsible for allergic reactions which can sometimes occur following treatment with the extracts of Tanacetum parthenium (Arch. Dermatol. Forsch. 1975, 251 (3):235-44; ibidem 1976, 255 (2):111-21; Contact Dermatitis, 1988, 38 (4):207-8; Am. J. Contact Dermatol. 1998-9 (1):49-50; Br. J. Dermatol. 1995, 132 (4): 543-7).

Extracts of Tanacetum parthenium containing parthenolide are disclosed in WO 94 06800; EP 0 553 658; WO 92 11857; GB 2,166,952; EP 98 041; WO 98 39018.

DISCLOSURE OF THE INVENTION

It has now been found that Tanacetum parthenium extracts substantially free from α-unsaturated γ-lactons, particularly from parthenolide, have interesting pharmacological properties together with a remarkably reduced risk of allergic reactions.

The extracts of the invention can therefore be conveniently used as active ingredients for the preparation of pharmaceutical or oral cosmetic compositions.

"Substantially free from α-unsaturated γ-lactons" and "substantially free from parthenolide" herein mean an extract having a weight content of parthenolide below 0.2%, preferably below 0.1%, more preferably below 0.09% and most preferably below 0.07%.

The extract of the invention is obtainable through a process which comprises:

a) extracting the plant (aerial parts) with acetone, alcohols or mixtures of these solvents with water;

b) extracting the material from step a) with hydrocarbons;

c) extracting the non-hydrocarbon phase with an apolar solvent;

d) treating the extract with the apolar solvent, previously evaporated and redissolved in water-alcoholic solution, with a strong basic resin;

e) eluting the resin with alcohols and removing the eluted solution;

f) treating the resin with alcoholic or water-alcoholic solutions of acids, concentrating the solution and extracting the resulting residue with an apolar solvent;

g) evaporating the apolar solvent from step f) to give a residue which is added to the residue from the evaporation of the hydrocarbon extract from step b) and to the acetonic or alcoholic phase obtained after the extraction with the apolar solvent of step c);

h) evaporating and drying.

Parthenolide and the other related sesquiterpene lactones can be removed in that the strong basic resin surprisingly does not cause the opening of the lactone ring, which is recognizedly sensitive to basic hydrolysis: the flavone components having acid hydroxy groups are therefore retained by the resin, contrary to parthenolide and related compounds which can therefore be removed.

The preferred solvents for the various extraction steps are reported in the following:

step a): acetone, methanol, ethanol or mixtures thereof with water;

step b): hexane, n-pentane, petroleum ether, ligroin;

step c): methylene chloride, chloroform, ethyl acetate, preferably methylene chloride;

step f): ethyl acetate.

"Alcoholic or water-alcoholic solvents" preferably means methanol or methanol with water percentages ranging from 10 to 80% by volume.

Basic resins which can be used according to the invention are commercially available, for example, under the registered names Relite 2A, Relite 3A2, Dowex 2.

Alternatively, the extract of the invention can be obtained starting from the conventional extracts commercially available, by treatment with strong basic resins, optionally previously extracting with hydrocarbon solvents the lipophilic components which could adversely interfere with the resin.

The content of α-unsaturated γ-lactons or of parthenolide in the final extract is determined by HPLC.

In the annexed Figure, the typical HPLC profile of the extract obtainable by the process described above is shown.

The extract of the invention has favourable pharmacological properties together with a remarkably reduced risk of inducing allergic reactions. The preservation of the pharmacological properties characteristic of the conventional extracts of Tanacetum parthenium, despite the almost complete removal of the sesquiterpene lactones, is even more surprising in view of the up-to-now documented reports as regards the relationship between bioactivity and composition of the known extracts (J. Pharm. Pharmacol. 1992,44:391-395.).

More particularly, the extracts substantially free from parthenolide of the invention have antiplatelet, antiinflammatory, analgesic, anti-migraine activities.

The invention therefore also relates to pharmaceutical compositions containing as active ingredient an effective amount of the Tanacetum parthenium extract of the invention in admixture with suitable carriers.

More particularly, the compositions of the invention will contain 10 to 500 mg of extract and will be administered 3-4 times a day, depending on the physician's prescription. The preferred administration route is the oral one, but other routes, such as the topical and parenteral, could be envisaged.

The compositions of the invention are particularly useful for the therapy and prophylaxis of migraine attacks.

The extracts of the invention can also be used as active ingredients for oral cosmetic formulations.

The invention is described in greater detail in the following examples.

EXAMPLE 1 – PREPARATION OF THE EXTRACT

Two kilograms of Tanacetum parthenium are extracted at 50-60°C with 20 L of 70% aqueous methanol. The extract is concentrated under vacuum to about 1 L and diluted with an equal volume of methanol. The resulting solution is extracted with 3 x 2 liters of n-hexane. The hexane extract is evaporated to dryness under vacuum to yield about 30 g of residue (extract H). The water-methanolic solution is then extracted with 2 x 0.5 L of methylene chloride. The organic phase is evaporated to dryness under vacuum to yield about 70 g of residues (extract DCM). The water-methanolic phase (extract HM) is set aside. The DCM extract is dissolved in 0.6 L of 90% methanol and treated under stirring with 0.6 L of strong basic resin (Relite 2A) for three-four hours. The

suspension is filtered under vacuum and the resin is washed with about 2 L of 90% methanol. The methanolic solution, containing parthenolide and its congeners, is removed. The basic resin is then treated under stirring for one hour with 0.6 L of methanol containing 65 ml of concentrated hydrochloric acid. The resin is filtered under vacuum and washed with a further 2.5 L of methanol. The filtrate and the washings are combined, concentrated under vacuum to about 200 mL and extracted with 3 x 200 ml of ethyl acetate. The resulting extract (E.A.), containing the flavone components tanetin and congeners, is evaporated to dryness under vacuum, to obtain about 4 g of a residue. The residues of the extracts H and E.A. are combined with the extract HM. The resulting solution is evaporated to dryness under vacuum and the solid residue is dried under vacuum at 50°C to constant weight. About 490 g of extract of departhenolidized T. parthenium are obtained which shows, by HPLC analysis, (column Zorbax SB C18; eluent H_2O + 0.01% TFA; B:MeCN + 0.01% TFA; gradient A:B:90%-10%:10%-90%; flow 1 ml/min), a parthenolide content below 0.07%.

EXAMPLE 2 - PHARMACOLOGICAL ACTIVITY

The experimental model of nitroglycerin-induced neuronal stimulation in the rat, described in Brain Research 1995,682:167-181; *ibidem* 1995 695,37-44 and in Neuropharmacol. 1997, 36(10);1417-1424, has been used.

Nitroglycerin (a vasodilator commonly used to treat some cardiovascular disorders) can be used as a diagnostic tool in the study of migraine headache, since its administration causes a spontaneous-like attack in migraieurs. The nitroglycerin-induced attack is not immediate, but delayed by some hours and typically

associated with the accompanying symptoms like nausea and photophobia. The vasodilating activity of organic nitrates, including nitroglycerin, arises from the metabolism to nitric oxide which is also reported to play a role as a neurotransmitter in the SNC. Neuronal stimulation rapidly activates the transcription of several proto-oncogenase, which, because of their rapid and transient induction, have been named immediate-early genes. Among them, *c-fos* is the most extensively studied and the protein encoded, Fos, is translocated to the nucleus where it persists for a few hours and can be detected immunoistochemically.

Experimental procedures

Twenty-five male Sprague-Dawley rats divided in 5 animal group each have been used. Two groups of animals (control and nitroglycerin) were injected with vehicle alone (0.5% carboxymethylcellulose, 10 ml/kg) while the 3 other groups were treated orally for 3 days with a Tanacetum parthenium extract substantially free from parthenolide (TPPDE) at the doses of 100 and 200 mg/kg and with Tanaceutum parthenium regular extract (TPRE) at the same dosages, respectively.

One hour after the last administration, all the animals, except the control group, were treated subcutaneously with 10 ml/kg of nitroglycerin.

Four hours after administration of nitroglycerin, the rats were anaesthetized with sodium pentobarbital and perfused with fixative through the ascending aorta. The brains were quickly removed and postfixed in the same fixative overnight. Sections through the entire brain were cut at 50 μm on a freezing sliding microtome. Sections were serially collected in six wells containing

cold phosphate buffered saline (PBS) and processed for immunoistochemistry as free-floating sections.

The results are reported in the following Table.

TABLE: Number of immunoreactive neurons (mean ± s.d.) in the paraventricular nucleus of the hypothalamus (PVH), locus coeruleus (LC), and parabrachial nucleus (PBN) 4 hours after nitroglycerin administration.

Treatment	PVH	LC	PBN
Control	95.4 ± 43.1	47.0 ± 21.2	27.3 ± 15.2
Nitroglycerin	574.2 ± 298.5	334.3 ± 61.4	231.3 ± 89.4
TPPDE (100) + nitroglycerin	378.5 ± 182.9*	200.5 ± 43.4*	140.7 ± 64.6*
TPPDE (200) + nitroglycerin	123.4 ± 74.5**	87.5 ± 24.5**	71.6 ± 28.6**
TPRE (200) + nitroglycerin	569.7 ± 288.3	321.2 ± 49.1	220.3 ± 87.5

*p<0.03 Test for TPPDE (100) + nitroglycerin vs. nitroglycerin control group

**p<0.001 Test for TPPDE (200) +nitroglycerin vs. nitroglycerin control group

Substitute sheet (Rule 26)

CLAIMS

1. Extracts of Tanacetum parthenium substantially free from α-unsaturated γ-lactons obtainable by a process which comprises:

a) extracting the plant (aerial parts) with acetone, alcohols or mixtures of these solvents with water;

b) extracting the material from step a) with hydrocarbons;

c) extracting the non-hydrocarbon phase with an apolar solvent;

d) treating the extract with the apolar solvent, previously evaporated and redissolved in water-alcoholic solution, with a strong basic resin;

e) eluting the resin with alcohols and removing the eluted solution;

f) treating the resin with alcoholic or water-alcoholic solutions of acids, concentrating the solution and extracting the resulting residue with an apolar solvent;

g) evaporating the apolar solvent from step f) to give a residue which is added to the residue from the evaporation of the hydrocarbon extract from step b) and to the acetonic or alcoholic phase obtained after the extraction with the apolar solvent of step c);

h) evaporating and drying.

2. An extract according to claim 1 substantially free of parthenolide.

3. An extract according to claim 1 or 2 having a content of α-unsaturated γ-lactons below 0.2%.

4. An extract according to claim 1 or 2 having a content of α-unsaturated γ-lactons below 0.1%.

5. Pharmaceutical compositions containing the extract of claims 1-4 in admixture with suitable carriers.

6. Oral cosmetic compositions containing as active ingredient the extracts of claims 1-4 in admixture with suitable carriers.