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(54) Title: METHOD FOR PREPARING A HYPERICUM EXTRACT IN NEEM OIL AND A SUBSTANCE SO OBTAINED

(57) Abstract: A method for making a Hypericum perforatum extract in Neem oil, provides of: combining an amount of Hypericum flowers and/or capsules with a quantity of Neem oil; - introducing into a reactor the mixture made of the amounts of Hypericum flowers and/or capsules and of Neem oil; maintaining the mixture vacuum, under agitation for a predefined time and temperature. A composition includes Neem oil and Hypericum flowers and/or capsules. Such composition includes moreover a solvent or a mixture of solvents.



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METHOD FOR PREPARING A HYPERICUM EXTRACT IN NEEM OIL AND A SUBSTANCE SO OBTAINED

TECHNICAL FIELD

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This invention relates to medical-surgical devices, in particular refers to a method for the preparation of a Hypericum perforatum extract (Hypericum) in Neem oil and to a substance so obtained.

10 BACKGROUND ART

It is known that the oleolito or the hypericum perforatum extract has anti-inflammatory effect, antiseptic and cicatrizing in the external wounds, ulcers and burns.

15 It is furthermore known that the Neem oil has cicatrizing, biocide, anti inflammatory, myasis insect repellent and not proprieties.

For example it is known in WO 2006/013607 that the oil mixture of Neem and the oily extract of Hypericum perforatum add the bioactive characteristics verified in the single components of
20 the mixture.

Said diluting effect precludes to think equivalent the biologic and biocide properties, of single obtainable compositive mixtures, because the biologic property is molecular, depends on the structure and depending on dose.

25

In such document WO 2006/013607, for indicated preparation modality, the hypericum oleolito cannot certainly satisfy the reproducibility criterion of the obtained oleolito results, considering the loss of the process conditions (temperature, time, lighting degree, agitation, drugs/oil ratio), to satisfy the criterion of a minimum reproducibility of the oleolito molecular composition.

30

Moreover in the preparation so obtained already at 16°C develops turbidity because of hydrocarbons, fat acids, etc. precipitation, so modifying the spreading and the use easiness of the preparation. This fact decreases the potentiality in the space-time, in relation to the climate.

35 Moreover the spagyric maceration used in the extraction process according to document

WO2006/013607 is subjected to climatic factor (light, temperature) variability and is subjected to process variable times.

DISCLOSURE OF THE INVENTION

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Object of this invention is to propose an industrial extraction method of the bioactive substances of *Hypericum perforatum* flowers and infructescences (capsules) using as extracting solution of the Neem oil without using other oil for the maceration.

10 Further object is to increase the extraction method for example by using a solvent.

An additional aim is to obtain *Hypericum* extracts directly in Neem oil concentrates and with greater applicability range in function of the temperature.

15 An additional aim is furthermore to offer a method highly easy and economically advantageous.

BEST MODE OF CARRYING OUT THE INVENTION

20 The method object of the present invention consists in putting the fresh, frozen, dried, lyophilized *Hypericum* flowers, and the infructescences (capsules), or their mixtures into a reactor, consisting of a steel container, equipped with central rotatory axis carrying, inserted on the axis, agitation blades, that can to be rotate with variable speed. The container carries an air-tight glass cover permeable to the radiations in the visible spectrum field.

25 The container is heated at vacuum state with depression not lower than 0,04 atmospheres.

The ratio value of solvent solution weight / flowers or capsules weight is between 3 and 1 and between 15 and 1 depending on the decreasing water content of flowers or capsules or the their mixtures. After flowers and/or capsule introduction, there is put the solvent chosen between:

30 a) Neem oil as which;

b) mixtures including Neem oil and a solvent or solvents mixtures, such as benzyl alcohol, ethanol 96°, phenylethyl alcohol, alcohol alphaphenylethyl, ethylic alcohol, isopropyl alcohol, octyl alcohol; glycols as glycerin, propylene glycol, polyethylenglycol; esters such as ethyl acetate, octyl acetate, ethyl lactate, isopropyl acetate, isopropyl myristate, isopropyl palmitate,
35 ethyl caprylate, ethylphenylacetate, ethyloleate etylinoate, ethylaurate, triethylcitrate,

phenylethyl acetate; triglycerides as: triolein, trilinolein, tributyrin, triacetin, tricapryline, tricaproine, tricapriline, paraffin oils, silicones, in ratio Neem oil – solvents between 1:2 and 1:0,01.

- 5 The listed extraction solvents at items a, b, alone or added with components such as silicones, paraffin oils, esters, triglyceride liquids at 4°C, can unfavorable precipitation phenomena in the topic composition during the preservation, widening the applicability range until temperatures close to 0°C or lower.
- 10 Therefore the vacuum is obtained, the heating of mass of the extraction solvent and biomass at temperatures ranging between 16°C and 100°C is activated and the helicoidal blades shaft is put into rotation.

The irradiation is activated and it is maintained the contact between extractant solution and
15 biomass for periods from 1 hour until 400 hours.

When the extraction time is elapsed, the maceration liquid is sent, interposing one or more filters, into dark glass amber colored container and therein the maceration product is preserved avoiding the contact with the air oxygen. The extraction kinetics is overseen monitoring, by
20 means of HPLC chromatography, the two molecules of marker hypericine and derivatives with 585 nanometers and the hyperphorine and derivatives with 278 nanometers.

With this method is obtained a composition as anti-inflammatory medicine, antiseptic and cicatrizing, for example in the treatment of outside wounds, ulcers, sores and burns, further
25 acting as repellent for myasis and not insects.

Further features will greatly result from the description of some practical realization preferred examples of the preparation method of the extract according to the invention, examples given hereinafter as indicative and not limitative.

30

Example 1

There are introduced in the reactor 300 grams of Hypericum flowers or Hypericum perforatum capsules dried, or frozen or lyophilized, etc. and vacuum is obtained after hermetic sealing of the glass cover of the reactor.

35

Therefore 4 kg of clear Neem oil are introduced, and when completed the solvent feeding, the multiple-blade agitator starts up, the temperature is grown to 50°C and there is radiation.

After 220 hour the mixing shaft is stopped, and simultaneous is blocked the heating and the radiation and the extract is transferred into an amber colored glass container.

One or two filtering cartridges for eliminating the suspension material are inserted in the adducing pipe of the extract. The Hypericum extract in Neem oil is preserved at dark at 20°C±1.

10 The extract yield was 2,85 kg equal to 71,25% of the introduced Neem oil.

Example 2

300 grams of lyophilized Hypericum flowers (about 2 liters of volume) of which 60% are infructescence are introduced into the reactor and 3,5 kg of extractant solution are added, consisting of Neem oil 41% (weight/weight), 26% of ethyl acetate, 16% of benzyl alcohol, 16% of ethanol at 96%.

The vacuum is obtained, it is programmed the heating to 35°C and the mixing speed is adjusted by fixing the rotation speed of the shaft supporting the blades, by radiating with white light lamps, for a total extraction time of 180 hours.

After such period, agitation and irradiation are stopped and the extract is transferred into amber colored glass container.

25

The recovered extract weight was of 2,8 kg equal to a yield of 80,3% of the extractant mixture.

Example 3

800 grams of Hypericum capsules or fresh Hypericum flowers are placed into reactor, both 3,8 kg of extractant solution of the following per cent composition: 50% of Neem oil, 15% of alcohol phenyl ethylic, 20% of propyl acetate, 10% of ethanol at 96°, 2% of isopropyl palmitate, 3% of paraffin oil with freezing point at - 20°C and the vacuum is obtained.

35 The heating is fixed to 65° C, the agitator is activated and, giving the irradiation, the extraction

is continued for 120 hours.

The agitation and the irradiation are stopped and the trial continues according to example 1 by transferring into dark glass container 2,3 kg of extract equal to a yield of 71,8%.

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An advantage is to increase the extraction method for example by using a solvent.

An additional advantage is to obtain Hypericum extract in Neem oil concentrates.

CLAIMS

- 1) Method for preparing a Hypericum perforatum extract in Neem oil, characterized by:
- combining an amount of Hypericum flowers and/or capsules with a quantity of Neem oil;
 - introducing into a reactor the mixture made of the amounts of Hypericum flowers and/or capsules and of Neem oil;
 - maintaining the mixture vacuum, under agitation for predefined time and temperature.
- 2) Method according to claim 1 characterized by providing moreover the irradiation of the mixture with light in the spectrum of the visible.
- 3) Method according to claim 1 characterized by maintaining between 1:3 and 1:15 the weight ratio between the amount of Hypericum flowers and/or capsules and the amount of Neem oil.
- 4) Method according to claim 1 characterized by using fresh, frozen, lyophilized or dried Hypericum flowers or capsules.
- 5) Method according to claim 1 characterized in that the predefined temperature is ranging between 16°C and 100°C.
- 6) Method according to claim 1 characterized in that the predefined time ranges between 1 hour and 400 hours.
- 7) Method according to claim 1 characterized in that the vacuum has a depression not lower than 0,04 atmospheres.
- 8) Method according to claim 1 characterized in that the agitation is variable between 2 and 30 rotations per hours.
- 9) Method according to any of the preceding claims characterized in that the amount of Neem oil consists of Neem oil and a solvent or a solvent mixture.
- 10) Method according to claim 9 characterized in that the Neem oil is at least 33% in weight of

the solvent or of the mixture of solvents.

- 11) Method according to claim 9 characterized in that the solvent is alcohol or glycols or esters or triglycerides or paraffin oils, or silicones or their mixtures.
- 5
- 12) Method according to claim 11 characterized in that the alcohol is of the type: benzyl alcohol, ethanol 96°, phenylethyl alcohol, alcohol alphaphenylethyl, ethylic alcohol, isopropyl alcohol, octyl alcohol.
- 10
- 13) Method according to claim 11 characterized in that the glycols are of the type: glycerin, propylene glycol, polyethylenglycol.
- 14) Method according to claim 11 characterized in that the esters are of the type: ethyl acetate, octyl acetate, ethyl lactate, isopropyl acetate, isopropyl myristate, isopropyl palmitate, ethyl caprylate, ethylphenylacetate, ethyloleate, ethyllinoleate, ethylaurate, triethylcitrate, phenylethyl acetate.
- 15
- 15) Method according to claim 11 characterized in that the triglycerides are of the type: triolein, trilinolein, tributyrin, triacetin, tricapryline, tricaproine, tricapriline.
- 20
- 16) Composition characterized by including Neem oil and Hypericum perforatum flowers and/or capsules.
- 17) Composition according to claim 16 characterized by further including a solvent or a mixture of solvents.
- 25
- 18) Composition according to claim 17 characterized in that the solvent is alcohol or glycols or esters or triglycerides or paraffin oils, or silicones or their mixtures.
- 30
- 19) Composition according to claim 18 characterized in that the alcohol is of the type: benzyl alcohol, ethanol 96°, phenylethyl alcohol, alcohol alphaphenylethyl, ethylic alcohol, isopropyl alcohol, octyl alcohol.
- 35
- 20) Composition according to claim 18 characterized in that the glycols are of the type: glycerin, propylene glycol, polyethylenglycol.

- 21) Composition according to claim 18 characterized in that the esters are of the type: ethyl acetate, octyl acetate, ethyl lactate, isopropyl acetate, isopropyl myristate, isopropyl palmitate, ethyl caprylate, ethylphenylacetate, ethyloleate etylinoleate, ethylaurate, triethylcitrate, phenylethyl acetate.
- 22) Composition according to claim 18 characterized in that the triglycerides are of the type: triolein, trilinolein, tributirina, tri-acetine, tricapriline, tricaproina, tricapriline.
- 23) Composition according to claim 17 characterized in that the Neem oil is at least 33% in weight of the solvent or the mixture of solvents.
- 24) Composition according to claim 17 characterized in that the use temperature of the composition as liquid lotion is lowered at least until 0°C.
- 25) Composition according to any of claims from 16 to 24 characterized in that the Hypericum flowers or capsules are fresh or frozen or dried or lyophilized or triturated before the maceration.
- 26) Composition according to any of claims from 16 to 24 characterized in that the weight ratio is variable between 1:3 and 1:15 among the Hypericum flowers or capsules and the Neem oil.
- 27) Use of the composition according to claims 16 to 26 for preparing a medicine.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/060104

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K36/38 A61K36/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/013607 A (CARNEVALI FIORELLA [IT]; VAN DER ESCH STEPHEN ANDREW [IT]) 9 February 2006 (2006-02-09) cited in the application abstract page 1, lines 1-9	16,25,27
Y	page 7, line 6 - page 10, line 16; figures 1-11	1-27
Y	EP 1 197 219 A (JUKUNDA NATURARZNEIMITTEL DR L [DE]) 17 April 2002 (2002-04-17) page 2, lines 3,4 page 2, line 55 - page 4, line 44; examples 1,2	1-27
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Further documents are listed in the continuation of Box C.

See patent family annex.

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- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
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- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *G* document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/060104

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KULEVANOVA S ET AL: "DETERMINATION OF TOTAL FLAVONOIDS AND QUERCETIN IN HYPERICI HERBA AND ITS AQUEOUS, AQUEOUS-ETHANOLIC AND OIL EXTRACTS" ACTA PHARMACEUTICA, ZAGREB, HR, vol. 50, no. 1, 1 January 2000 (2000-01-01), pages 29-37, XP009033700 ISSN: 1330-0075 abstract page 29, paragraph 2 - page 30, paragraph 3 page 30, paragraph 7 - page 31, paragraph 1; tables I-III</p>	1-27
Y	<p>FR 2 864 447 A (CRISPU JEAN MARIE [FR]) 1 July 2005 (2005-07-01) abstract page 1, line 4 - page 2, line 22</p>	1-27
Y	<p>"FINAL REPORT ON THE SAFETY ASSESSMENT OF HYPERICUM PERFORATUM EXTRACT AND HYPERICUM PERFORATUM OIL" INTERNATIONAL JOURNAL OF TOXICOLOGY, TAYLOR AND FRANCIS, WASHINGTON, DC, US, vol. 20, no. SUPPL. 02, 1 January 2001 (2001-01-01), pages 31-39, XP009016121 ISSN: 1091-5818 page 32, left-hand column, paragraph 2-4 page 33, left-hand column, paragraph 4 - page 34, left-hand column, paragraph 3</p>	1-27
A	<p>EP 0 415 327 A (KAO CORP [JP]) 6 March 1991 (1991-03-06) abstract page 2, line 50 - page 3, line 43; examples 1-5</p>	1-15,27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2008/060104

Patent document cited in search report	A	Publication date	Patent family member(s)	Publication date
WO 2006013607	A	09-02-2006	AT 381936 T	15-01-2008
			AU 2005268407 A1	09-02-2006
			BR PI0514092 A	27-05-2008
			CA 2571647 A1	09-02-2006
			CN 101014352 A	08-08-2007
			DE 602005004030 T2	21-08-2008
			EP 1773365 A2	18-04-2007
			ES 2299062 T3	16-05-2008
			JP 2008509133 T	27-03-2008
			<hr style="border-top: 1px dashed black;"/>	
EP 1197219	A	17-04-2002	AT 314080 T	15-01-2006
			DE 10050574 A1	02-05-2002
<hr style="border-top: 1px dashed black;"/>				
FR 2864447	A	01-07-2005	NONE	
<hr style="border-top: 1px dashed black;"/>				
EP 0415327	A	06-03-1991	DE 69005059 D1	20-01-1994
			DE 69005059 T2	09-06-1994
			ES 2062232 T3	16-12-1994
			JP 2544239 B2	16-10-1996
			JP 3163026 A	15-07-1991
<hr style="border-top: 1px dashed black;"/>				