EXTRACTS OF HYPERICUM PERFORATUM AND FORMULATIONS CONTAINING THEM

The present invention relates to an extract of Hypericum perforatum (St.-John's-wort) obtainable by fractioning water-alcoholic, alcoholic or acetonic extracts of the plant with esters of water-immiscible C_1-C_5 alcohols, and the formulations containing it.

Flowering tops of Hypericum perforatum contain a number of classes of structurally different substances acting directly or indirectly on central nervous system.

Hypericum perforatum is in fact known to contain a series of active compounds such as hypericin, hyperforin and dimeric flavones which exert antidepressive and anxiolytic activities on animals and humans. The action mechanisms of these compounds are different: anti-MAO action, action on serotonin release, as it will be described in detail in the following, and activity on the benzodiazepine receptors. The activity of hypericin has been discussed for a long time due to the contrasting data reported in literature.

The controversial antidepressive activity of Hypericin has recently confirmed in pharmacological models *in vivo*. It has, in fact, been proved that hypericin is active when administered in the presence of dimeric procyanidins contained in the extracts of Hypericum perforatum (45th Annual Congress of the Society for Medicinal Plant Research, September 7th-12th, 1997, Regensburg, Germany, V. Butterwecke et al., Abstract No. 011).

Hyperforin has recently been the object of numerous studies which made it possible to establish its

important role as antidepressant; studies carried out by the Applicant proved that this molecule has serotonin-mimetic activity. Other components considered important are dimeric flavones deriving from apigenin which are looked upon as natural benzodiazepines, as reported in "Naturally Occurring Benzodiazepines Structure, distribution and function", I. Izquierdo and J. Medicine Eds., 1993, page 33.

These products, and particularly hyperforin, are not very stable in the usual conditions of extraction and conservation; according to WO 97/13489 (Schwabe), the hyperforin content in a water-alcoholic extract of St.-John's-wort falls nearly to zero already after a few weeks.

According to WO 97/13489, in order to obtain stable extracts with a constant content in hyperforin, extraction, purification and conservation should be carried out in the presence of antioxidants such as vitamin C and the esters thereof, sulfurated amino acids, etc.

EP 0599307 (Schwabe) discloses the removal of hypericin, responsible for undesired photo-sensitizing effects, by means of polyvinylpyrrolidone and other chemicals; said Patent describes extracts with a hyperforin content of at least 5% obtained without using antioxidants.

Comparative pharmacological and clinical data between methanolic conventional extracts or those according to the monograph of Commission E, and the extracts prepared according to EP 0599307 and WO 97/13489, are not available. On the other hand, the conventional extract of Hypericum perforatum

recognizedly contains large amounts of flavonoids, which are potent radical scavengers and therefore natural stabilizing agents for easy-to-oxidize substances, together with other substances which can significantly contribute to the activity of the extract.

It has now been found stable, particularly active extracts of Hypericum perforatum, containing the main compounds responsible for the pharmacological activity, in particular hypericin, hyperforin, flavonoids and xanthones, can be prepared by a process which comprises:

a) extraction of flowering tops of Hypericum perforatum with alcohol or acetone;

b) filtration of the extracts and concentration thereof;

c) dilution of the concentrate from step b) with water or water/alcohol mixtures;

d) extraction of the aqueous mixture from step c) with aliphatic esters;

e) filtration and evaporation to dryness of the ester extracts from step d); and, optionally,

f) solubilization in a solution of an organic acid in aqueous ethanol and evaporation of the solvent at a temperature below 40°C.

The process of the invention is preferably effected carrying out the first extraction (step a) with methanol or ethanol, in drug:solvent weight/volume ratios ranging from 1:2 to 1:20, preferably from 1:2 to 1:10, at temperatures ranging from room temperature to the reflux temperature of the solvent, preferably between room temperature and 40°C.

Aliphatic esters for use in step c) are, for example, ethyl acetate, methyl acetate, butyl acetate;

ethyl acetate being most preferred.

The extraction by means of said esters is carried out after treatment of the concentrated alcoholic or acetonic extract with an equal volume of water or with alcohol/water mixtures in volume ratios ranging from 1:2 to 1:5.

The volume ratio of aqueous mixture to ester is not critical and can range within wide limits, but it usually ranges from 1:0.5 to 1:2.

The extraction is preferably carried out repeatedly, generally at least three times, using fresh aliquots of solvent.

Optional step f) is effected dissolving the concentrate from e) in a solution of an organic acid such as citric, malic, acetylaspartic or phosphoric acids in 95% ethanol.

The resulting extract of the invention, analyzed according to M. Brolis et al., J. of Chromatography, 825 (1998), 9-16, contains hyperforin in amounts ranging from 5 to 20% by weight when using spontaneous vegetable biomasses, whereas hyperforin contents from 10 to 50% by weight are obtained when using selected vegetable biomasses. In both cases, the content in total hypericins is higher than 0.5%, whereas dimeric flavones are present in amounts from 1 to 2% by weight. This wide content variability depends on the time at which the plant is collected, the seed content in the flowering tops and the amount of stems present in the biomass.

This extract, compared to the total extract, has surprisingly a higher activity in various pharmacological models used for the evaluation of the antidepressive and anxiolytic effects and is stable in

time without further specific treatments.

Furthermore, the process of the invention provides stable extracts with no need for further processing. The extracts should anyway be shielded from light to avoid photodegradations.

The resulting extract has the following activity on the inhibition of serotonin (5-HT) and dopamine (DA) uptake.

Table I - Effect of the extract of Hypericum perforatum prepared according to Example 1 in inhibiting ^{3}H 5-HT and ^{3}H DA uptake.

	IC_{50} µg/ml	
Substances	^{3}H DA	^{3}H-5 HT
Alcoholic extract	4.05 ± 0.93	28.0 ± 1.7
Hexane extract	0.86 ± 0.02	3.08 ± 0.62
Example 1	0.32 ± 0.04	2.72 ± 1.1
Hyperforin	1.54 ± 0.23	4.75 ± 0.79
Hypericin	> 50	> 50
Pseudo-hypericin	1.40 ± 0.13	27.0 ± 1.10

These data clearly evidence that the extract according to the invention has a potency several times higher than hyperforin and other known extracts.

In the in vivo tests, the extract of the invention has shown a higher activity than known products, mainly consisting of alcoholic, methanolic, hexane extracts, with or without hypericin; moreover, the extract has proved a higher reproducibility and stability of action in time. As in vivo test to verify the antidepressive effect, were selected the escape deficit development test and the inhibition of the ethanol consumption in Sardinia alcohol preferring rats according to models known in literature.

In the escape deficit development test, the extract of the invention has surprisingly shown a higher activity than the known extracts and an activity comparable with that of known medicaments such as imipramine. In this test, rats are fastened and subjected to mild, short, unavoidable electric shocks for 50 min (pre-test). Twenty-four hours later, animals are tested for their ability to avoid the same stimuli on their tails, in a situation in which escape is impossible. A rat on the average makes 26 escapes out of 30 stimuli (naive controls), whereas an animal subjected to pre-test only makes 1-3 escapes (ED controls). Hyporeactivity induced by the pre-test does not take place in rats pre-treated for 1-3 weeks with antidepressants such as imipramine or fluoxetine. The St.-John's-wort extracts orally administered to rats one hour before exposure to the unavoidable stress cause an increase in reactivity to the escape test, which is enhanced when pre-treatment is effected for 1-2 weeks.

Table II: Antidepressive effect of extracts of Hypericum perforatum and fractions thereof in rats in the escape test with a 2 week- pre-test.

Substances	Dose/mg/kg	Number of escapes
Hypericum alcoholic extract	1000	16.6 ± 2.8
Hexane extract	600	17.2 ± 1.6
Example 1	200	23.3 ± 0.4
Example 1	100	18.3 ± 0.2
Example 1	50	13.3 ± 0.4
Controls ed	--	1.6 ± 0.1
Naive controls	--	24.1 ± 0.1

Statistical analysis: Kruskal-Wallis non parametric ANOVA KW = 13.462 p = 0.0012

Hypericum alcoholic extract vs naive	p<0.01
Hypericum extracted Ex. 1 200 mg/kg vs naive	n.s.
Naive vs AND	p<0.01

In the test of the reduction of alcohol consumption in Sardinia rats (which is an index of depression and anxiety) according to procedures known in literature, the extracts of the invention induce, after three-day administration, a 75% decrease in alcohol consumption in favour of water compared with controls, whereas the reduction in alcohol consumption after treatment with the methanolic or hexane extract is respectively 30 and 40%.

The extract of the invention can be included in formulations for the oral use, such as ready-to-use solutions, soft- or hard- gelatin capsules, tablets and controlled-release tablets. The dosages of extract in the formulations range from 10 to 100 mg per dose in the usual formulations and up to 300 mg in the controlled-release formulations, in this case the preferred dose being 300 mg per dose daily.

The examples reported hereinbelow illustrate the invention in greater detail.

Example 1: Preparation of a dry extract of Hypericum perforatum standardized in the active components.

4 Kg of *Hypericum perforatum* flowering tops are extracted with 4 x 15 L of methanol in an extraction plant of 25 L capacity. The combined methanol extracts are concentrated to 2.5 L under vacuum; the concentrate is diluted with an equal volume of water, then counter-

extracted with 3 x 1.5 L each of ethyl acetate. The organic phase is filtered and concentrated to dryness under vacuum. The residue is dissolved in a solution of 2 g of citric acid in 1.3 l of 95% ethanol. The organic phase is then evaporated to dryness under vacuum at a temperature not above 40°C to obtain kg 0.32 of a brown-yellow extract containing 20% of hyperforin, 0.9% of total hypericins (hypericin plus pseudohypericin) and 1% of diapigenin.

Example 2: Preparation of an extract of Hypericum perforatum standardized in the active components.

60 kg of *Hypericum perforatum* flowering tops, collected and mechanically dried at a temperature not above 60°C, are extracted under mild reflux with 4 x 20 volumes of acetone. The combined extracts are filtered to remove the biomass residues, then concentrated to dryness under vacuum to obtain 3 kg of extract containing 0.4% of total hypericins and about 25% of hyperforin. This extract is suspended in 10 L of a methanol/water 3:7 mixture and counter-extracted with butyl acetate to complete exhaustion in polyphenols, checking exhaustion by thin layer chromatography on silica gel, eluting with ethyl acetate/methanol/H_2O 100:13.5:10. The aqueous phase is removed, whereas the organic one is dried over Na_2SO_4 and concentrated to dryness under vacuum at a temperature not above 40°C, to obtain 1.6 kg of dry extract containing 0.7% of total hypericins, about 40% of hyperforin and 1.4% of dimeric flavones.

Example 3: Preparation of a multicomponent extract of Hypericum perforatum, standardized in the active components.

60 kg of *Hypericum perforatum* flowering tops, collected and mechanically dried at a temperature not above 60°C, are extracted continuously with 98% methanol until exhaustion of extractables; the methanolic extract is concentrated to 30 L and the concentrate is diluted with an equal volume of water, filtering off insolubles which separate during dilution; the clear solution is extracted with 3 x 30 L each of water-saturated ethyl acetate, then dried over Na_2SO_4 and the solvent is removed under vacuum to obtain kg 3.8 of a brown extract containing 25% of hyperforin, 1.2% of total hypericins and 1.2% of dimeric diflavones.

Example 4: Solution containing the lipophilic extract of *Hypericum perforatum* prepared according to Example 1.

Extract of Hypericum perforatum prepared according to Example 1	10.0 g
Ammonium glycyrrhizinate	0.5 g
Propylene glycol	35.0 g
70% Sorbitol solution	25.0 g
Purified water	q.s. to 100 ml

Example 5: Coated tablets containing the extract of Hypericum perforatum prepared according to Example 2.

Extract of Hypericum perforatum prepared according to Example 2	300.00 mg
Soy polysaccharides	54.75 mg
Lactose	46.00 mg
Cross-linked sodium carboxymethylcellulose	40.00 mg
Silica	20.00 mg
Polyvinylpyrrolidone	5.00 mg
Talc	2.50 mg
Magnesium stearate	1.75 mg

Coating:	
Hydroxypropyl methylcellulose	10.00 mg
Talc	8.50 mg
Titanium dioxide	5.00 mg
Triacetin	2.00 mg
Polysorbate 80	0.50 mg
Red iron oxide	1.00 mg

Example 6: Soft-gelatin capsules.

Extracts of Hypericum perforatum of Example 1	100 mg
Saccharose monopalmitate	100 mg
Polyethylene glycol 400	220 mg
Glycerin	15 mg
Purified water	15 mg

CLAIMS

1. A process for the preparation of extracts of Hypericum perforatum which comprises:

a) extraction of flowering tops of Hypericum perforatum with alcohol or acetone;

b) filtration of the extracts and concentration thereof;

c) dilution of the concentrate from step b) with water or water/alcohol mixtures;

d) extraction of the aqueous mixture from step c) with aliphatic esters;

e) filtration and evaporation to dryness of the ester extracts from step d); and, optionally,

f) solubilization in a solution of an organic acid in aqueous ethanol and evaporation of the solvent at a temperature below 40°C.

2. A process as claimed in claim 1 wherein the extraction of step a) is carried out in methanol or ethanol.

3. A process as claimed in claim 1 wherein the extraction of step a) is carried out in acetone.

4. A process as claimed in claim 1 wherein the dilution of step c) is carried out in methanol or ethanol.

5 A process according to any one of claims 1 to 4, in which the aliphatic esters used in step d) are selected from ethyl acetate, methyl acetate, butyl acetate.

6. A process as claimed in claim 5 in which ethyl acetate is used.

7. A process as claimed in claim 1 wherein solubilization of optional step f) is carried out in

organic acids selected from citric, malic, acetylaspartic or phosphoric acids in solution in 95% ethanol.

8. Extracts of Hypericum perforatum obtainable by the processes of claims 1-7.

9. Extracts as claimed in claim 8 having a hyperforin content ranging from 10 to 50% by weight, a total hypericins content higher than 0.5% by weight and a content in dimeric flavones from 1 to 2% by weight.

10. Pharmaceutical compositions containing as active ingredient an extract of claims 8 or 9.

11. The use of the extracts of claims 8 or 9 for the preparation of antidepressive medicaments.



20000720 4-1

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau IPOS

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 35/78 — A1

(11) International Publication Number: WO 99/64027

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/EP99/03881

(22) International Filing Date: 4 June 1999 (04.06.99)

(30) Priority Data:
(1) MI98A001311 10 June 1998 (10.06.98) IT

(71) Applicant *(for all designated States except US)*: INDENA S.P.A. [IT/IT]; Viale Ortles, 12, I–20139 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BOMBARDELLI, Ezio [IT/IT]; Via Val di Sole, 22, I–20141 Milano (IT). GABETTA, Bruno [IT/IT]; Viale Ortles, 12, I–20139 Milano (IT). MORAZZONI, Paolo [IT/IT]; Viale Ortles, 12, I–20139 Milano (IT).

(74) Agent: MINOJA, Fabrizio; Bianchetti Bracco Minoja s.r.l., Via Rossini, 8, I–20122 Milano (IT).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

1 PD
1 appln
3 INVS.

IPC : A61K 35/78
IPER 30/12/99.

(54) Title: EXTRACTS OF *HYPERICUM PERFORATUM* AND FORMULATIONS CONTAINING THEM

(57) Abstract

An extract of *Hypericum perforatum* obtained by fractioning water–alcoholic, alcoholic or acetonic extracts of the plant with water–immiscible esters of C_1–C_5 alcohols.