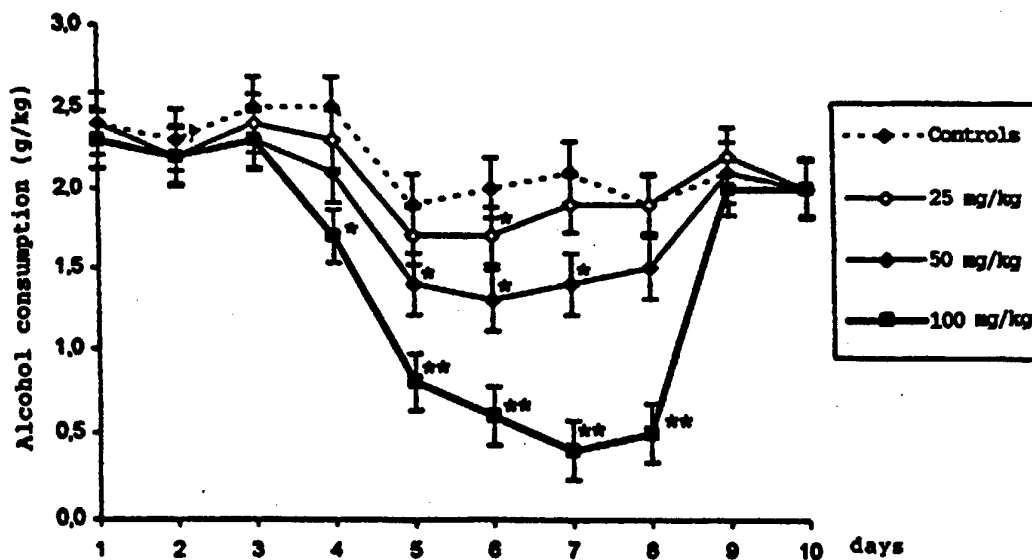




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(21) International Application Number: PCT/EP96/01916 (22) International Filing Date: 8 May 1996 (08.05.96) (30) Priority Data: MI95A000958 12 May 1995 (12.05.95) IT (71) Applicant (for all designated States except US): INDENA S.P.A. [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): MORAZZONI, Paolo [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT). BOMBARDELLI, Ezio [IT/IT]; Viale Ortles, 12, I-20139 Milano (IT). (74) Agent: BIANCHETTI, Giuseppe; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).	(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, 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.	

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF ALCOHOL ADDICTION



(57) Abstract

The present invention relates to pharmaceutical compositions for the treatment of alcohol addiction, characterized in that they contain - as the active principle - a purified lipophilic extract of Salvia miltiorrhiza Bunge.

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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF ALCOHOL
ADDICTION

The present invention relates to pharmaceutical compositions for the treatment of alcohol addiction, characterized in that they contain - as the active principle - a purified lipophilic extract of Salvia miltiorrhiza Bunge. The invention also relates to a process for the preparation of the purified lipophilic extract of Salvia miltiorrhiza Bunge, and the extract thus obtained. Furthermore, the invention relates to the use of the pure active principles contained in this extract (Tanshinone IIA and Miltirone) in the treatment of alcohol addiction and to pharmaceutical compositions for said treatment which contain as the active principles Tanshinone IIA and/or Miltirone.

Alcohol abuse and alcohol addiction, phenomena which can be collectively referred to by the term alcoholism, represent a serious problem for the whole of modern society (Gessa G.L., Bisogno compulsivo di bere e "principio del piacere" [The compulsive need to drink and the pleasure principle] in *Medicina delle tossicodipendenze* [Drug Addiction Medicine] II, 5 (1994)). In Italy, for example, more than 9% of the population (about 5 million people) are heavy drinkers and more than 1 million people are alcohol-addict (Calamo-Specchia F.P. - Epidemiologia dell'alcolismo in Italia [Epidemiology of alcoholism in Italy] in *Atti del VII Congresso Nazionale della S.I.A.*, [Records of the 7th National Congress of the S.I.A.] Mediserve, Rome, 295-301, (1991)). These figures become much higher if we

consider countries such as the United States of America where there are more than 13 million alcohol-addicts. Alcohol abuse and actual alcohol addiction result in an enormous outlay of public money (recently, since 1991, in the United States about 200 thousand million dollars a year have been spent) and are the cause of enormous social and psychological damage for the individuals involved.

The existing approaches for the treatment of alcoholism, in addition to those of a psychological nature (group therapy, etc.), consist in the use of drugs such as disulfiram and calcium carbamide which act on the metabolism of alcohol, inhibiting hepatic aldehyde-dehydrogenase and therefore raising the hematic levels of acetaldehyde, with all the undesirable phenomena which occur each time ethanol is taken.

According to the present state of the art, the sole plant whose derivatives have been used for the treatment of alcoholism is the Pueraria lobata (Radix puerariae), which is widely used in traditional Chinese medicine and forms the subject of Patent Application WO 93/00896.

It has now been surprisingly found that the purified lipophilic extract of the roots of Salvia miltiorrhiza Bunge can be used with success for reducing the voluntary consumption of alcohol and other drugs which induce addiction. Furthermore, individual pure products isolated from the extract, such as Tanshinone IIA and Miltirone, have also proved to be useful. The dried roots of Salvia miltiorrhiza Bunge (Danshen, Radix Salviae miltiorrhizae) are officially listed in the Chinese Pharmacopeia; they are widely used for the

treatment of menstrual disturbances, cardiovascular diseases such as, for example, angina pectoris and abnormalities of platelet function, as well as insomnia (Tang, W. Eisenbrand, G., Salvia miltiorrhiza Bge., in Chinese drugs of plant origin; Chemistry, Pharmacology, and Use in Traditional Chinese Medicine, pp. 891-902 - Springer-Verlag, Berlin (1992); Kee Chang Huang, Antianginal Herbs in The Pharmacology of Chinese Herbs, pp.81-84 - CRC Press, Boca Raton (1993)).

10 The use of hydro-alcoholic extracts of Salvia miltiorrhiza for the topical treatment of excess fat deposits is also known (Italian Patent 1,239,281).

15 The extract according to the invention, unlike that which is known, is of the lipophilic type and is obtained by extraction of the roots of Salvia miltiorrhiza with acetone, at temperatures of between about 20 and 50°C, using a drug/solvent ratio (w/v) of between 1:2 and 1:10, preferably about 1:5. Following concentration down to about 1/20th to 1/50th of the initial volume, water and a solvent which is immiscible in water are added. The acetone is removed by means of repeated washings with water while the organic phase containing the active principles is concentrated, following dilution, where necessary, with 95° ethanol.

25 The lipophilic extract according to the present invention is characterized by having a content of from 5 to 30% w/w, preferably from 15 to 25%, of Tanshinone IIA and of from 0.5 to 3% w/w, preferably of from 0.8 to 2%, of Miltirone.

30 Determination of the inhibiting action on alcohol consumption was performed using alcohol-consuming rats

of the strain called "Sardinian alcohol-preferring" (Sp)
(Fadda F., Mosca E., Colombo G., Gessa G.L., Alcohol-
preferring rats: Genetic sensitivity to alcohol-induced
stimulation of dopamine metabolism, in *Physiol. Behav.*
5 47, 727 (1990)).

These animals, which given a free choice between
alcohol and water consume daily 6 to 7 g of alcohol per
kg of body weight (with a water-to-alcohol ratio higher
than 2:1), during the last few years have been used with
10 success to determine the effect of various substances on
the voluntary consumption of alcohol; see, for example,
Balakleevsky A., Colombo G., Fadda F., Gessa G.L., Ro
19-4603, a benzodiazepine receptor inverse agonist,
attenuates voluntary ethanol consumption in rats
15 selectively bred for high ethanol preference, in *Alcohol*
Alcohol. 25, 449-452 (1990); Fadda F., Garau B., Colombo
G., Gessa G.L., Isradipine and other calcium channel
antagonists attenuate ethanol consumption in ethanol-
preferring rats, in *Alcoholism: Clinical and*
20 *Experimental Research* 16(3), 449-452 (1992).

The animals, which were kept under normal housing
conditions, were given a free choice between water
(which was always present) and alcohol (a 10% solution
v/v) which was offered for a period of 4 hours a day
25 (i.e. the first 4 hours of darkness during the day/night
cycle). The amounts of water and alcohol consumed were
recorded every day at the same time. Food was offered
ad libitum. Once a stable consumption of alcohol and
water was reached, different doses of purified
30 lipophilic extract of Salvia miltiorrhiza (prepared as
above) dissolved in dimethylsulfoxide, were administered

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orally in a volume amount of 2 ml/kg once a day for 5 consecutive days. An equal volume of the carrier was used as the control. At the end of treatment, the alcohol and water consumption was recorded until the values recorded prior to the treatment were re-established. Statistical significant difference from the mean of the values obtained in the group treated with the carrier alone (* $p < 0.05$, ** $p < 0.01$) was evaluated by the Dunnet test for multiple comparisons.

Figure 1 shows the effect of repeated oral administration of increasing doses of the extract on alcohol consumption; Figure 2 shows the effect of increasing doses on water consumption. Figure 3, on the other hand, shows the effect on alcohol consumption resulting from repeated doses both of the extract and of the principles contained therein (Tanshinone IIA and Miltirone); Figure 4, finally, illustrates the effect on water consumption of the extract and the said principles when administered in repeated doses.

From an examination of Figures 1 and 2 it can be concluded that the extract reduces alcohol consumption significantly and in a dose-related manner, this result being achieved in some cases (i.e. with the highest dose) already after the first day of administration. The reduction in alcohol consumption remains constant until the fifth day and then regresses following suspension of the treatment. Moreover, it is surprising to note that this trend is accompanied by the tendency for a gradual increase in the water consumption, as if the animal were substituting it for the alcohol. This latter observation is of particular importance since it

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shows that the treatment carried out with the product being tested is perfectly well-tolerated and the animal returns without any difficulty to a more physiological life cycle, using water instead of alcohol.

5 The administration in constant doses, repeated for 5 days, of both the extract and Tanshinone IIA and Miltirone (Figures 3 and 4) confirms the results indicated above, but demonstrates that the pure components - taking account of the doses - are the main
10 factors responsible for the effect of the extract.

 The invention therefore provides pharmaceutical compositions which can be administered orally and which contain as the active principle either the lipophilic extract of Salvia miltiorrhiza or the purified active
15 principles Tanshinone IIA and/or Miltirone, the latter being obtainable from commercial sources or by means of conventional purification of the lipophilic extract. The compositions of the invention, in addition to conventional excipients or carriers, will contain from
20 about 10 to about 500 mg of the extract or the equivalent doses of Tanshinone IIA and Miltirone, taking into account their extract content.

EXAMPLE 1

Preparation of the lipophilic extract

25 1 kg of finely ground roots of Salvia miltiorrhiza are extracted 4 times using 5 l of acetone at 50°C. The recombined extracts are concentrated under a vacuum to 500 ml; the concentrate is diluted with 1 l of methylene chloride and 500 ml of H₂O. The phases are separated
30 and the organic phase containing the active principles is washed with water until the acetone and the unwanted

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polar substances are eliminated.

The chloromethylene phase is concentrated to a small volume; the residue is diluted with 300 ml of 95° ethanol. The solution is concentrated under a vacuum to dryness at a temperature of 60°C. After drying overnight, 11.3 g of extract are obtained, containing 15.2% of Tanshinone IIA and 1.1% of Miltirone.

EXAMPLE 2

Formulation in capsule form

10	Each 130 mg capsule contains:	
	Extract of Example 1	25 mg
	Pregelatinized starch	25 mg
	Microcrystalline cellulose	49 mg
	Lactose	15 mg
15	Colloidal silica	6 mg
	Cross-linked sodium carboxymethylcellulose	6.5 mg
	Polyvinylpyrrolidone	2.5 mg
	Magnesium steareate	1 mg

EXAMPLE 3

Formulation in tablet form

20	Each 400 mg tablet contains:	
	Extract of Example 1	100 mg
	Pregelatinized starch	100 mg
	Microcrystalline cellulose	96 mg
25	Lactose	45 mg
	Colloidal silica	25 mg
	Cross-linked sodium carboxymethylcellulose	20 mg
	Polyvinylpyrrolidone	10 mg
	Magnesium steareate	4 mg

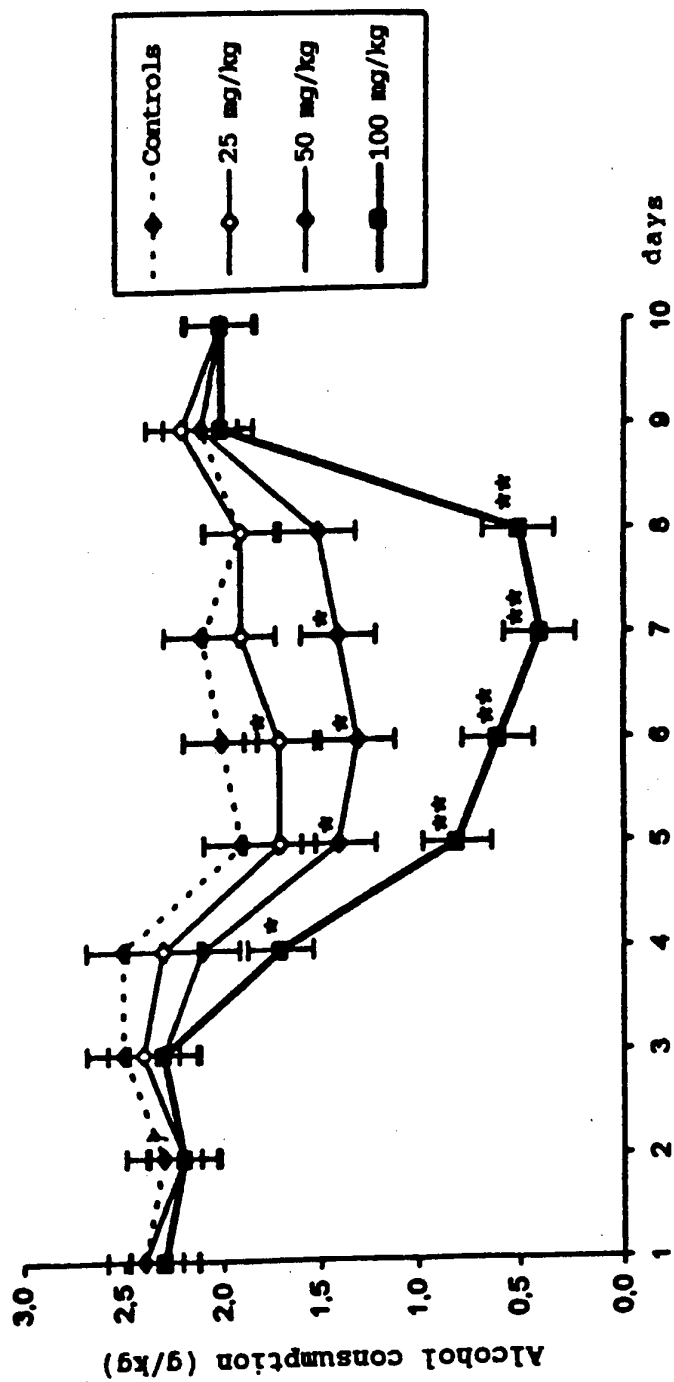
CLAIMS

1. Lipophilic extract of the roots of Salvia miltiorrhiza Bunge characterized in that it contains
5 from 5 to 30% w/w of Tanshinone IIA and from 0.5 to 3% w/w of Miltirone.
2. An extract according to claim 1, containing from 15 to 25% w/w of Tanshinone IIA.
3. An extract according to claim 1 or 2, containing
10 from 0.8 to 2% of Miltirone.
4. A process for the preparation of the extract of claims 1-3, comprising the extraction of the roots of Salvia miltiorrhiza with acetone, subsequent concentration, treatment of the concentrate with water
15 and a water-immiscible solvent, removal of the acetone by washing with water and concentration of the organic phase.
5. Lipophilic extract of Salvia miltiorrhiza Bunge obtainable by extraction of the roots of Salvia miltiorrhiza with acetone, subsequent concentration,
20 treatment of the concentrate with water and a water-immiscible solvent, removal of the acetone by washing with water and concentration of the organic phase.
6. Oral pharmaceutical compositions containing the
25 extract according to Claims 1-3 or 5 in admixture with suitable carriers.
7. Composition according to Claim 6 in the form of capsules or tablets.
8. Use of the extract according to Claims 1-3 or 5 for
30 the preparation of medicaments for the treatment of alcohol addiction.

9. Use of Tanshinone IIA for the preparation of medicaments for the treatment of alcohol addiction.
10. Use of Miltirone for the preparation of medicaments for the treatment of alcohol addiction.
- 5 11. Oral pharmaceutical compositions containing as the active principle Tanshinone IIA and/or Miltirone in admixture with suitable carriers.

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FIGURE 1



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FIGURE 2

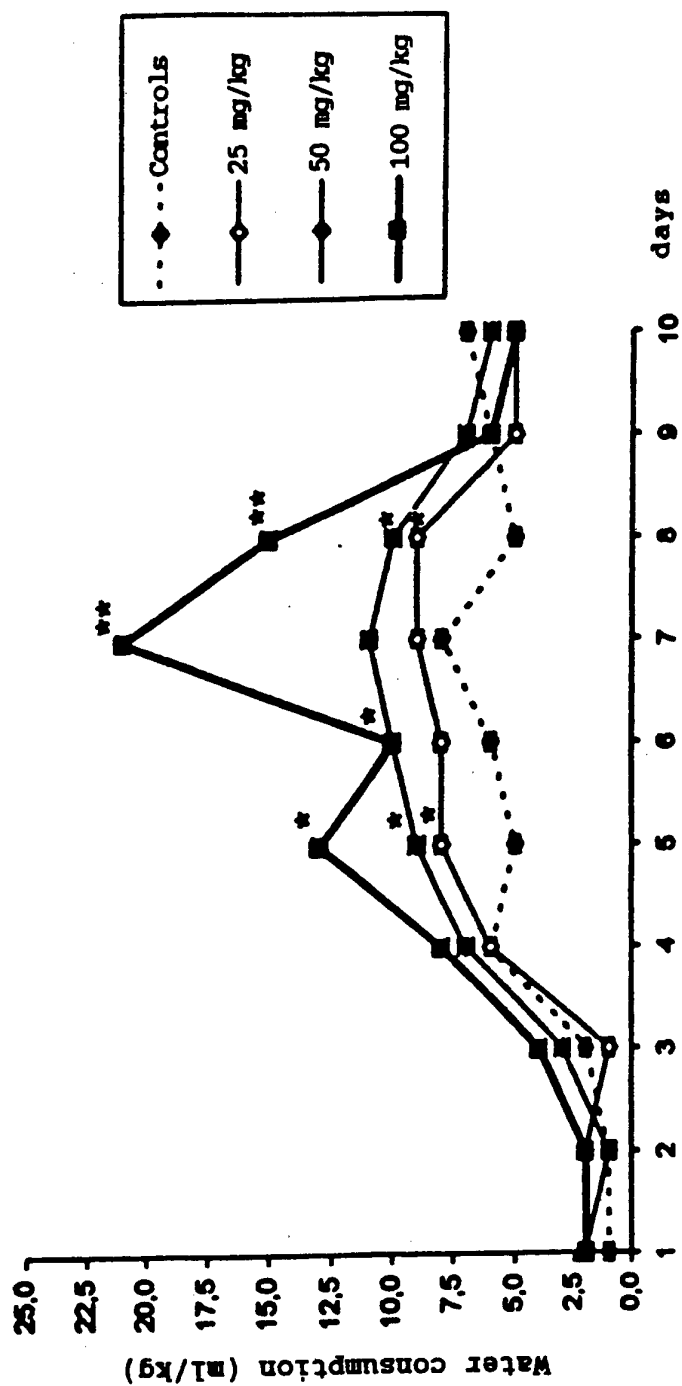
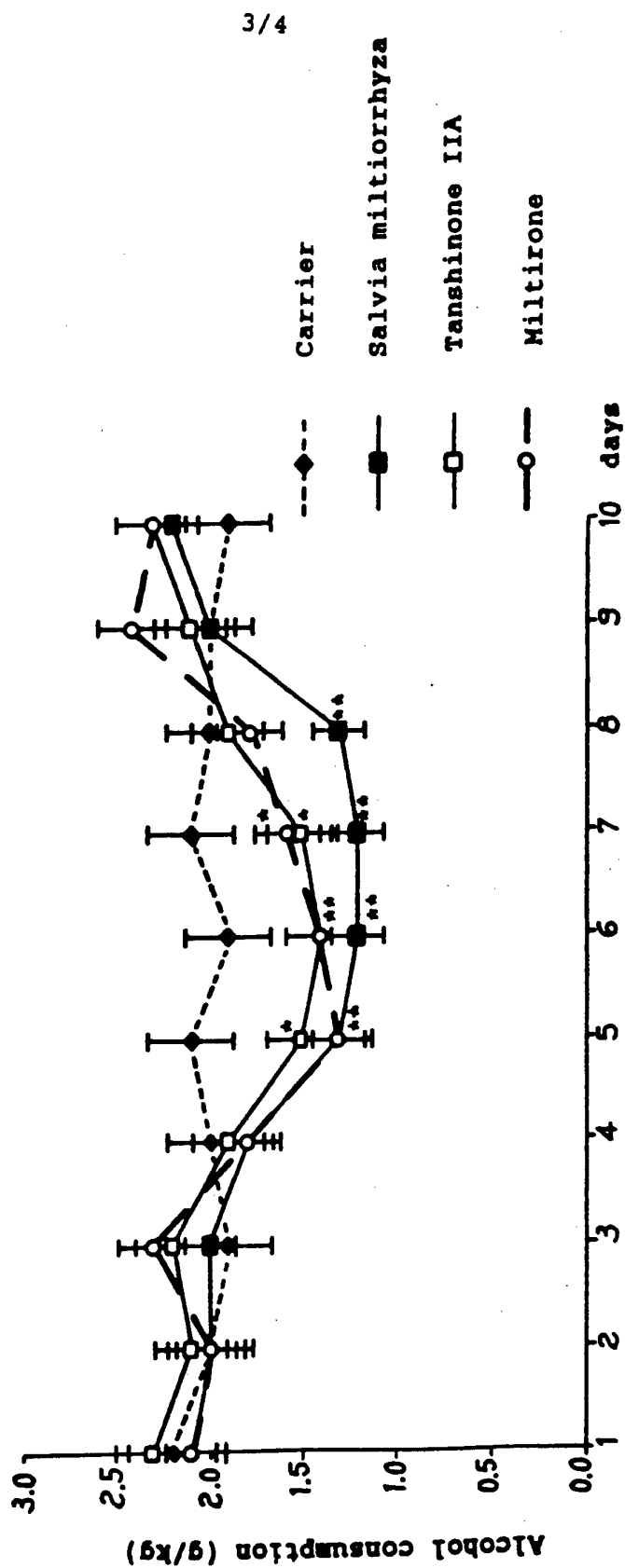
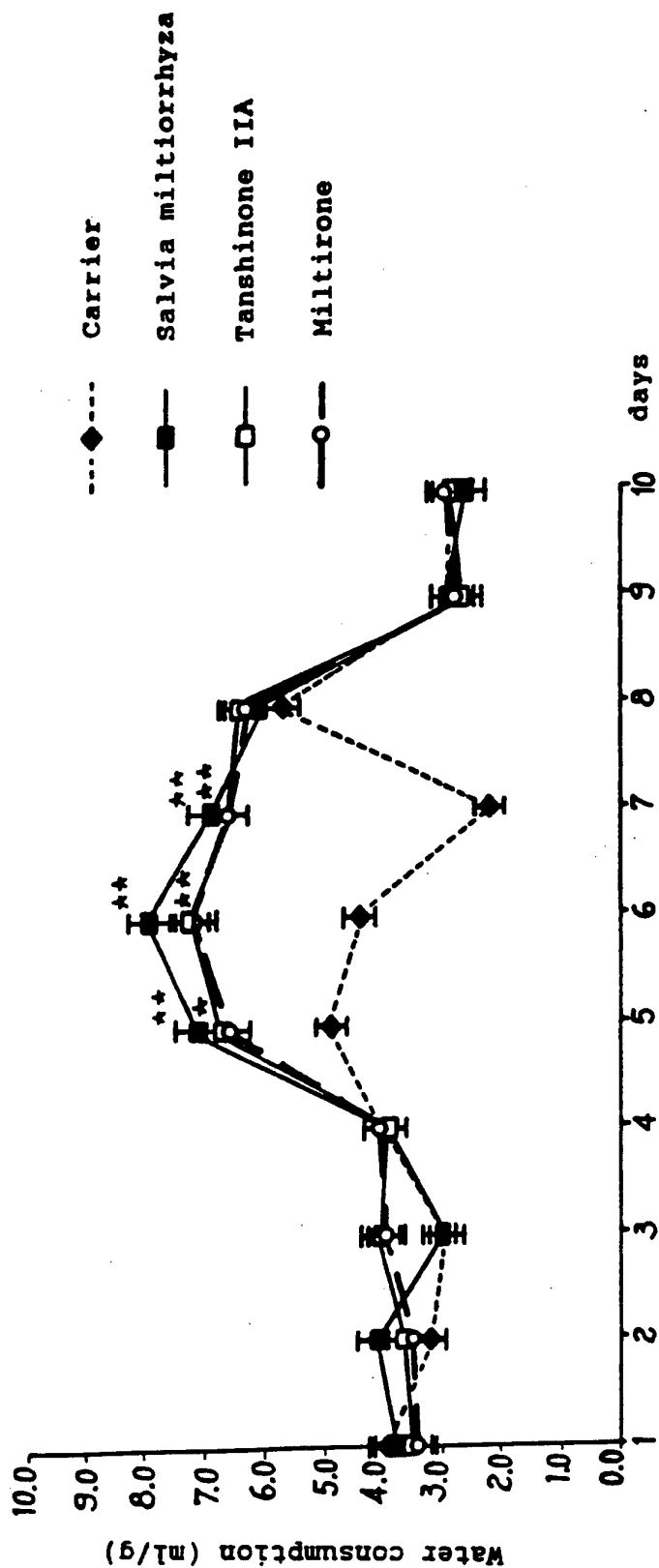


FIGURE 3



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FIGURE 4



INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/EP 96/01916

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K35/78 A61K31/34 A61K31/12

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)

IPC 6 A61K

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

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 427 026 (INDENA SPA) 15 May 1991 see page 4, line 5-7; example 2 ---	1-3,5
X	CHEMICAL ABSTRACTS, vol. 117, no. 9, 1992 Columbus, Ohio, US; abstract no. 86739, WENG X.C. ET AL: "Antioxidant activity of quinones extracted from tanshen (Salvia miltiorrhiza Bunge)" page 429; column 1; XP002012181 see abstract & J.Agric.Food Chem. 1992 40(8), 1331-1336 --- -/--	1-3,5

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Inten. Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE NAPRALERT STN AN92:5003, 1974 LI, C.P.: "CHINESE HERBAL MEDICINE" XP002012182 see abstract & Chinese herbal medicine U.S.Dept.Health, Education and Welfare Publ.No.(NIH)75-732 Washington, D.C.	11
X	--- NEUROSCIENCE LETTERS, vol. 127, 1991, pages 237-241, XP000601484 CHI-MING LEE ET AL: "Miltirone, a central benzodiazepine receptor partial agonist from a Chinese medicinal herb Salvia Miltiorrhiza" see the whole document	11
Y	---	9,10
Y	MERCK & CO. INC.: "THE MERCK MANUAL OF DIAGNOSIS AND THERAPY" 1992, MERCK RESEARCH LABORATORIES XP002012180 see pages 1551-1555, especially pages 1554-1555 "treatment"	9,10
A	--- WO,A,93 00896 (THE ENDOWMENT FOR RESEARCH IN HUMAN BIOLOGY INC) 1993 see page 19 -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Application No
PCT/EP 96/01916

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		FI-A- 935954	14-02-94
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