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(71) Applicant: **GIELLEPI S.P.A.** [IT/IT]; Via Mascheroni 4, I-20123 Milano (IT).

(72) Inventors: **MUGELLI, Alessandro**; Via Sant'Ansano 7, I-50014 Fiesole (IT). **KARIOTI, Anastasia**; Kalynoy 12, Halandri, GR-15231 Athens (GR). **BILIA, Annarita**; Via Il Pino 27, I-59015 Carmignano (IT). **DI CESARE MANNELLI, Lorenzo**; Via Cesare Balbo, I-59100 Prato (IT). **FIRENZUOLI, Fabio**; Via Vasari 32, I-59013 Montemurlo (IT). **GALLO, Eugenia Rosaria**; Via Giovanni Fattori 24, I-59100 Prato (IT). **GHELARDINI, Carla**; Via Bastianaccio 21, I-51100 Pistoia (IT). **PACINI, Alessandra**; Via S. Ammirato 10, I-50136 Firenze (IT). **VANNACCI, Alfredo**; Via Molino di Filettole 8, I-59100 Prato (IT). **ZANARDELLI, Matteo**; Via Franco Fornari 37, I-29122 Piacenza (IT).

(74) Agents: **GERVASI, Gemma** et al.; Corso di Porta Vittoria 9, I-20122 Milano (IT).

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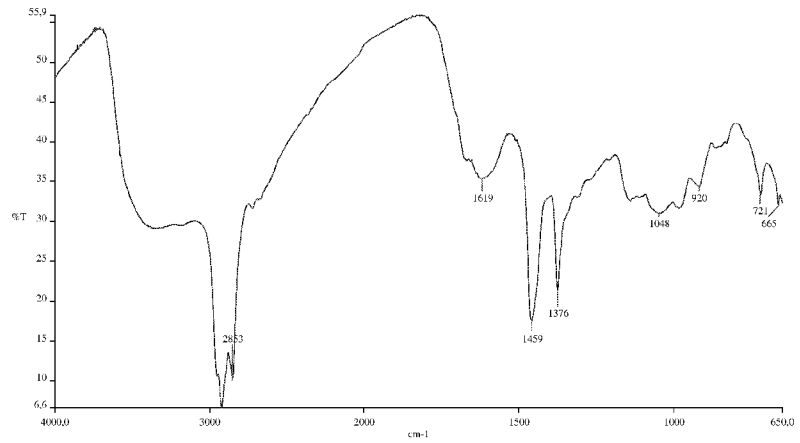
(54) **Title**: EXTRACTS OF ASTRAGALUS MEMBRANACEUS, THEIR PREPARATION AND USE AS ANTIHYPERALGESIC AND ANTIALLODYNIC DRUGS

Fig. 1



(57) **Abstract**: Herein are described hydroalcoholic extracts of *Astragalus membranaceus*, their preparation and use as antihyperalgesic and antiallodynic drugs.

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Extracts of *Astragalus membranaceus*, their preparation and use as antihyperalgesic and antiallodynic drugs

Field of the invention

The present invention relates to the field of plant product extracts.

Background of the invention

The International Association for the Study of Pain (IASP) defines neuropathic pain as: "An unpleasant sensory and emotional experience that is associated with actual or potential tissue damage or otherwise described as such". It is a major problem in neurology as it is frequent and often debilitating on account of its chronic nature.

The characteristics of this pain vary from patient to patient, but in general there are continuous burning sensations or electric shocks; paraesthesias, i.e. abnormal sensations in the areas surrounding the primary location of the pain, are often present. Examples are post-herpetic pain, phantom limb pain, which can occur following an amputation, pain in peripheral neuropathies such those present in diabetes or in AIDS, pain in the so-called complex regional syndromes or reflex sympathetic dystrophies and pain from lesions of the central nervous system. The latter can be sequelae of a stroke, of a trauma, of tumours or due to systemic diseases. In recent years, interest has focused on the neuropathic pain induced by chemotherapeutic drugs (oxaliplatin, paclitaxel, etc.).

In particular, oxaliplatin is a third generation platinum-organic compound that induces the appearance of a neuropathic syndrome characterised by paraesthesia and dysesthesia of the extremities accompanied by cramps. These symptoms occur in 85-95% of patients treated. While the acute toxicity disappears within a few days of administration, repeated treatment induces a chronic form that does not subside in the interval between the treatment cycles (Gamelin et al., Semin Oncol 29, 2002; Extra et al. Semin Oncol 25, 1998; Andre et al., J Clin Oncol 17, 1999; Cersosimo Ann Pharmacother 39, 2005). An accumulation of the drug in the ganglia of the dorsal roots seems to be the first phenomenon at the basis of the development of the neuropathy (Ta et al., Neurotoxicology 27: 992-1002, 2006).

Oxaliplatin induces two types of peripheral neuropathy, an acute and a chronic type both characterised by hyperalgesia and allodynia until there is impairment of daily activities. This painful symptomatology is the main reason for suspension of antitumor therapy (Extra et al., 1998; Andre et al., 1999; Gamelin et al., 2002; Cersosimo et al., 2005).

Even taxane-based antineoplastic therapy contemplates a distal, symmetrical, axonal, predominantly sensory neuropathy among the side effects (Argyriou, et al., Critical Reviews in Oncology/Hematology 66:218-228, 2008). Some epidemiological data reports an onset of peripheral neuropathy in 60% of patients taking taxanes. Even in this case, the symptoms most frequently reported by patients include paraesthesia and motor peripheral neuropathies that determine pain in distal segments of the limbs.

Classic analgesics (NSAIDS, tramadol and morphine) and also anti-epileptics (gabapentin and pregabalin) are only partially effective in treating neuropathic syndromes caused by the administration of chemotherapeutic drugs and there are no compounds to date capable of completely controlling this type of increased pain perception (Albers et al. Cochrane Database Syst Rev CD005228, 2011; Kaley & Deangeli, 2009). The neuropathic pain, being difficult to treat, is therefore one of the most frustrating problems of analgesic therapy.

Is thus clear, in the light of the foregoing, the interest in availing of different drugs so as to be able to intervene on this type of pain without the above drawbacks.

Brief description of the drawings

Figure 1 shows the IR spectrum of the extract according to the invention

Figure 2 shows the spectrum obtained by nuclear magnetic resonance technique

Figure 3 shows the profile of the two-dimensional HETCOR spectrum (called HMQC).

Summary of the invention

Hydroalcoholic extracts of *Astragalus membranaceus* , useful as antihyperalgesic and antiallodynic drugs, are described.

Detailed description of the invention

It has now been surprisingly found that extracts of *Astragalus membranaceus* are useful for overcoming the above problems.

Astragalus membranaceus is a plant belonging to the Fabacease family that has powerful antioxidant properties (Sheng et al., Chin. Med. J. 5:43-49, 2005, Li et al., Urol. Res. 34:277-282, 2006, Luo et al. Phytother. Res. 23:761-767, 2009) of which the dried root is used.

The extracts according to the invention are hydroalcoholic extracts substantially obtained following the methodologies known in this field.

Water or alcohol/water mixtures can be used as alcohols for the extraction; ethanol is one example of alcohol that can be used for the extraction.

The amount of alcohol is normally between 0 - 80% (calculated in volume with respect to the total volume of the mixture), preferably 70%.

Extraction is carried out at room temperature by maceration and under stirring for a few days in order to obtain an exhaustive extraction of the phytocomplex, possibly by renewing the solvent employed one or more times. Alternatively the extraction can be performed by percolation with hydroalcoholic solutions as defined above or for decoction.

The alcohol is subsequently evaporated and the aqueous solution is lyophilized thus obtaining the desired product.

For the sake of completeness and clarity, an example of hydroalcoholic extraction according to the invention, is set out below.

Example 1

Preparation of a hydroalcoholic extract of *Astragalus membranaceus*

100 grams of dried root and finely powdered *Astragalus membranaceus* root are macerated in 500 ml of an ethanol/water solution (70% v/v of ethanol) by extracting under stirring by prolonged maceration (for example two days); the solvent is changed and the extraction continued until exhaustive extraction (extraction takes place 3 or 4 times) of the phytocomplex.

The resulting solutions are pooled and the ethanol is evaporated with a rotary evaporator at low pressure. The resulting aqueous solution is lyophilized thus obtaining a white-pale yellow powder that is analysed by various methods to obtain a fingerprint.

In particular, the powder obtained was analysed by infrared, by the nuclear magnetic resonance technique thus respectively obtaining the spectra set out in Figure 1 and 2.

Further is shown the profile of the two-dimensional HETCOR spectrum called HMQC, which also represents the fingerprint of the extract in question (see Figure 3).

In the first technique, the sample is prepared by mixing with nujol to obtain a preparation of semisolid consistency which under IR analysis has the following fingerprint:

-in the area between 3600-3100 (peak at 3370 cm^{-1}) cm^{-1}, a wide band resulting from stretching vibrations of O-H bonds, can be observed

-a 2900-2850 cm^{-1} two narrow bands, resulting from stretching vibrations of C-H bonds, can be observed

-a 1460 cm^{-1} a narrow band, resulting from vibrations of stretching vibrations of bonds C-O, can be observed

-1200-1000 cm^{-1} characteristic region of vibrations resulting from stretching vibration rings of the bonds (C-OH) and vibration of the bonds (C-O-C) → 1139

-1049: attributed to arabinofuranose and ramnopyranose

In the case of analysis by nuclear magnetic resonance, the extract is dissolved in dimethyl sulfoxide deuterate (50 mg extract/ml solvent), as can be seen in Figure 2:

- the majority of the signals are present in the area between 3.0-4.1 ppm carbinolic protons of the saccharide units

- at 5.3 ppm the anomeric protons of the saccharide units can be observed.

In addition, the profile of the two-dimensional HETCOR spectrum called HMQC shows that:

- The anomeric proton at 5.3 ppm has a cross-peak with an anomeric carbon ~ 92.0 ppm

- The group of carbinolic proton signals in the area between 3 and 4 ppm has characteristic cross-peaks with the carbons falling within the area between 60.0 and 82.0 ppm, which correspond to the carbinolic carbons of the saccharides.

The signals at 60.0 ppm belong to the free methylenes (-CH2), while the signals at 82.0 ppm belong to the carbons on the positions of bonds in the polysaccharide chain.

The lyophilized extract thus obtained can then be formulated for administration, preferably in oral form and, if necessary, can see the addition of the usual excipients used in this type of formulation such as for example inorganic excipients (such as for example silica gel) or excipients of a polysaccharide nature (such as for example: maltodextrin or lactose).

PHARMACOLOGICAL RESULTS

In rats, the repeated administration over three weeks of the hydroalcoholic extract of *Astragalus membranaceus* solubilised in carboxymethylcellulose 1% at a dose of 100 mg and 300 mg kg^{-1} p.o. proved capable of preventing the onset of the reduction of the pain threshold caused by treatment with oxaliplatin at a dose of 2.4 mg kg^{-1} i.p. for 21 days (5 days per week). In particular, full restoration of the normal pain threshold can be observed at the higher dose. At the reported doses, the extract under analysis proved to be effective in rats, both in conditions of hyperalgesia (paw-pressure) as shown in the below Table,

Tab. 1a.

EFFECT OF EXTRACTS OF ASTRAGALUS MEMBRANACEUS IN HYPERALGESIA INDUCED IN RATS BY OXALIPLATIN (PAW PRESSURE TEST)			
	Pressure (g)		
TREATMENT	TREATMENT		
		Dose	day 21
CARRIER	CMC		68.5 ± 1.8
OXALIPLATIN	CMC		39.2 ± 1.6§
CARRIER	HYDROALCOHOLIC EXTRACT	300	64.8 ± 2.3
OXALIPLATIN	AQUEOUS EXTRACT	100	50.8 ± 1.6^
OXALIPLATIN	AQUEOUS EXTRACT	300	52.7 ± 2.2*
OXALIPLATIN	ALCOHOLIC EXTRACT	100	50.0± 1.1^
OXALIPLATIN	ALCOHOLIC EXTRACT	300	53.6 ± 0.6*
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	30	41.2 ± 2.8
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	100	55.8 ± 1.1*
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	300	67.9 ± 2.1*
Oxaliplatin was administered at a dose of 2.4 mg kg^{-1} i.p. for 5 consecutive days per week and for 3 weeks (cumulative dose of 36 mg kg^{-1}). Oxaliplatin was dissolved in a 5% glucose solution. Extracts of *astragalus membranaceus* were suspended in carboxymethylcellulose (1%; CMC) and administered p.o. daily for 21 days from the first day of treatment with the chemotherapeutic drug. The animals in the control groups were treated with the carrier. The test was conducted on day 21. § $P<0.01$ compared against treatment with carrier/CMC; ^ $P<0.05$ and * $P<0.01$ with respect to the rats treated with oxaliplatin/CMC. Each value represents the average obtained from the evaluation of 12 rats.			

In both the allodynia ones in the presence of both a mechanical stimulus (Von Frey) and thermal stimulus (cold plate) as respectively shown in the following Tables 1b and 1c

Tab. 1b

EFFECT OF EXTRACTS OF ASTRAGALUS MEMBRANACEUS IN OXALIPLATIN-INDUCED ALLODYNIA INDUCED IN RATS (VON FREY TEST)			
	Pressure (g)		
TREATMENT	TREATMENT		
		Dose	Day 21
CARRIER	CMC		23.7 ± 1.2
OXALIPLATIN	CMC		13.8 ± 0.9§
CARRIER	HYDROALCOHOLIC	300	23.4 ± 1.3
OXALIPLATIN	AQUEOUS EXTRACT	300	16.3 ± 1.0
OXALIPLATIN	ALCOHOLIC EXTRACT	300	18.2 ± 0.7^
OXALIPLATIN	HYDROALCOHOLIC	30	13.5 ± 1.1
OXALIPLATIN	HYDROALCOHOLIC	100	18.6 ± 0.8^
OXALIPLATIN	HYDROALCOHOLIC	300	21.8 ± 1.2*

Tab. 1c

EFFECTS OF EXTRACTS OF ASTRAGALUS MEMBRANACEUS IN OXALIPLATIN-INDUCED ALLODYNIA IN RATS (COLD PLATE TEST)			
	Retraction latency (s)		
TREATMENT	TREATMENT		
		Dose	Day 121
CARRIER	CMC		24.1 ± 1.4
OXALIPLATIN	CMC		16.2 ± 0.8§
CARRIER	HYDROALCOHOLIC	300	24.0 ± 1.2
OXALIPLATIN	AQUEOUS EXTRACT	300	17.3 ± 1.0
OXALIPLATIN	ALCOHOLIC EXTRACT	300	17.5 ± 1.1
OXALIPLATIN	HYDROALCOHOLIC	30	19.1 ± 1.0^
OXALIPLATIN	HYDROALCOHOLIC	100	21.6 ± 1.0*
OXALIPLATIN	HYDROALCOHOLIC	300	23.6 ± 1.3*

Oxaliplatin was administered at a dose of 2.4 mg kg^{-1} i.p. for 5 consecutive days per week and for 3 weeks (cumulative dose of 36 mg kg^{-1}). Oxaliplatin was dissolved in a 5% glucose solution. The animals in the control groups were treated with the carrier. The test was conducted on day 21. § $P<0.01$ compared against treatment with carrier/CMC; ^ $P<0.05$ and * $P<0.01$ with respect to the rats treated with oxaliplatin/CMC. Each value represents the average obtained from the evaluation of 12 rats.

These same tables also show how an antihyperalgesic action, albeit characterised by lesser effectiveness, is also performed by the aqueous and alcoholic extract of *Astragalus membranaceus*. Only the alcoholic extract maintains a significant efficacy against mechanical allodynia (Tab. 1b), while neither the alcoholic nor the aqueous extract are able to exert a protective effect towards the thermal allodynia (Tab. 1c).

The 30 mg kg^{-1} p.o. dose of hydroalcoholic extract only exerts its pain threshold increasing action in the cold plate test. It is interesting to observe that the hydroalcoholic extract of *Astragalus membranaceus* does not induce analgesia per se in the absence of a painful condition (Tab. 1a, 1b, 1c).

The hydroalcoholic extract is also capable of improving the motor coordination of rats subjected to treatment with oxaliplatin.

In the following Table 1d

Tab. 1d

EFFECT OF THE HYDROALCOHOLIC EXTRACT OF ASTRAGALUS MEMBRANACEUS IN MOTOR IMPAIRMENT INDUCED IN RATS BY OXALIPLATIN.			
	Number of falls		
TREATMENT	TREATMENT		
		Dose	day 21
CARRIER	CMC		0.67 ± 0.33
OXALIPLATIN	CMC		4.42 ± 0.47§
CARRIER	HYDROALCOHOLIC EXTRACT	300	0.74 ± 0.2
OXALIPLATIN	AQUEOUS EXTRACT	300	3.89 ± 0.51
OXALIPLATIN	ALCOHOLIC EXTRACT	300	3.40 ± 0.33
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	30	3.23 ± 0.55
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	100	2.98 ± 0.25*
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	300	2.45 ± 0.45*

	Balancing time (s)		
TREATMENT	TREATMENT		
		Dose	day 21
CARRIER	CMC		600 ± 0
OXALIPLATIN	CMC		199 ± 25§
CARRIER	HYDROALCOHOLIC EXTRACT	300	600 ± 0
OXALIPLATIN	AQUEOUS EXTRACT	300	185 ± 19
OXALIPLATIN	ALCOHOLIC EXTRACT	300	345 ± 39^
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	30	185 ± 19
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	100	369 ± 49*
OXALIPLATIN	HYDROALCOHOLIC EXTRACT	300	548 ± 71*

Oxaliplatin was administered at a dose of 2.4 mg kg^{-1} i.p. for 5 consecutive days per week and for 3 weeks (cumulative dose of 36 mg kg^{-1}). The oxaliplatin was dissolved in a 5% glucose solution. The extract of *astragalus membranaceus* was suspended in carboxymethylcellulose (1%; CMC) and administered p.o. daily for 21 days from the first day of treatment with the chemotherapeutic drug. The animals in the control groups were treated with the carrier. The test was conducted on day 21. § $P<0.01$ compared against treatment with carrier/CMC; ^ $P<0.05$ and * $P<0.01$ with respect to the rats treated with oxaliplatin/CMC. Each value represents the average obtained from the evaluation of 12 rats.

It can indeed be seen that in the rota-rod test the rats that were also, throughout treatment with oxaliplatin, co-administered hydroalcoholic extract at the doses of 100 and 300 mg kg^{-1} p.o., show a better motor coordination with respect to those only treated with the chemotherapeutic drug. This effect is not only expressed with a reduction in the number of falls from the rotating rod but also with an increase in the balancing time. The dose of 30 mg kg^{-1} p.o., is also active on this last parameter. (Tab. 1d).

The alcoholic extract at the dose of 300 mg kg^{-1} p.o. significantly increases the time spent on the rotating rod even if with less efficacy with respect to the hydroalcoholic extract. On the contrary, the aqueous extract shows no effectiveness with respect to the oxaliplatin-induced motor alterations (Tab. 1d).

EFFECT OF THE HYDROALCOHOLIC EXTRACT OF *ASTRAGALUS MEMBRANACEUS* IN PACLITAXEL-INDUCED HYPERALGESIA IN RATS.

(PAW-PRESSURE TEST)

	Pressure (g)		
TREATMENT	TREATMENT		
		Dose	Day 18
CARRIER	CMC		69.1 ± 3.2
PACLITAXEL	CMC		34.2 ± 2.2§
PACLITAXEL	HYDROALCOHOLIC EXTRACT	300	50.5 ± 4.1*

Paclitaxel was administered at a dose of 1 mg kg^{-1} i.p. for 4 days (days 1,3,5,8). The cumulative dose is 4.0 mg kg^{-1}. Paclitaxel was suspended in a mixture of 10% Cremophor in saline solution. The test was performed on day 18. The extract of *Astragalus membranaceus* was suspended in carboxymethylcellulose (1%; CMC) and administered p.o. daily for 18 days starting from the first day of treatment with the chemotherapeutic drug. The animals in the control groups were treated with the carrier. § $P<0.01$ compared with treatment with carrier/CMC; * $P<0.01$ with respect to rats treated with paclitaxel/CMC. Each value represents the average obtained from the evaluation of 6 rats.

Tab. 2 shows the antihyperalgesic effect of the hydroalcoholic extract under analysis at the dose of 300 mg kg^{-1} p.o. on paclitaxel-induced hyperalgesia evaluated in rats in the paw pressure test.

An analogous result was obtained by the same extract at a dose of 300 mg kg^{-1} p.o. in the presence of a reduction of the pain threshold in streptozotocin-induced pain in an animal model of diabetes. See Tab. 3.

EFFECT OF THE HYDROALCOHOLIC EXTRACT OF *ASTRAGALUS MEMBRANACEUS* IN STREPTOZOTOCIN-INDUCED HYPERALGESIA IN RATS.

(PAW-PRESSURE TEST)

	Pressure (g)		
TREATMENT	TREATMENT		
		Dose	*Day 21*
SALINE	CMC		57.4 ± 4.5
STZ	CMC		35.7 ± 4.2§
STZ	Hydroalcoholic	300	48.2 ± 3.5*

Streptozotocin (STZ) was administered at a dose of 50 mg kg^{-1} i.v. (single injection, in saline solution) 21 days before the test. The extract of *astragalus membranaceus* was suspended in carboxymethylcellulose (1%; CMC) and administered p.o. daily for 21 days from the day of treatment with STZ. The animals in the control groups were treated with the carrier. § $P<0.01$ compared with treatment with saline/CMC; * $P<0.01$ with respect to rats treated with STZ/CMC. Each value represents the average obtained from the evaluation of 6 rats.

This is in line with what is written in the introduction, since the oxidative damage is a common factor in both neuropathies from chemotherapeutic drugs that the metabolic diseases such as diabetes where the hyperglycaemia translates into an increased production of mitochondrial superoxide radical with consequent increased exposure of cells to ROS (Lowell & Shulman 2005).

The hydroalcoholic extract of Astragalus membranaceus in animals is free of antihyperalgesic effect as regards some neuropathic pains such as for example pain induced by antiviral agents, loose ligation of the sciatic nerve and by experimental osteoarthritis induced by monoiodoacetate.

It is effective in reversing, in the test animals, oxaliplatin-induced neuropathic pain, it does not alter the viability of the HT-29 cells treated with the same chemotherapeutic drug, which allows exclusion of its causing the survival of tumour cells by invalidating the action of oxaliplatin.

In the context of the study, we have focused attention on a tumoural line of human colon carcinoma cells (neoplasia that is particularly sensitive to oxaliplatin) where we induced the damage with the chemotherapeutic agent under analysis. The value of IC_{50} exercised by oxaliplatin in this cell type was evaluated in the presence and in the absence of the extract of hydroalcoholic preparation. Testing by means of cell viability assay. The treatment has not altered viability.

The administration of 6 g kg^{-1} of hydroalcoholic extract does not alter, in rats, the motor coordination evaluated by means of the rota-rod and the spontaneous motility test.

The histopathological analysis carried out on liver and kidney samples from rats subjected to co-treatment with oxaliplatin and hydroalcoholic extract of Astragalus membranaceus has shown an appreciable protective effect on the part of the plant extract under analysis in respect of the alterations consequent to the cytotoxic effect of the chemotherapeutic drug. In particular, at the level of the kidney, the pathological picture indicative of the presence of acute crescentic and/or focal segmental glomerulonephritis caused by treatment with oxaliplatin, is significantly reduced by the administration of the hydroalcoholic extract of *Astragalus membranaceus*. Similarly, in the liver, the acute inflammatory process arising as a result of metabolic damage caused by the presence of the chemotherapeutic drug, damage that primarily manifests as ballooning degeneration, appear strongly reduced by treatment with the hydroalcoholic extract of *Astragalus membranaceus* which is therefore also an effective protector against drug-induced hepatotoxicity.

The administration of the hydroalcoholic extract of Astragalus membranaceus at a dose of 300 mg/kg p.o. once/day, in conjunction with oxaliplatin, has shown to have a statistically significant protective effect, in respect to the damage induced by chemotherapeutic drugs, at the level of the basal ganglia attached to the posterior roots. This effect is evidenced by alterations of the nuclei and of the nucleoli of the ganglion neurons. In particular, the increase in the number of multinucleated neurons and of neurons whose nucleolus occupies an eccentric position, and significantly reduced by co-treatment with the hydroalcoholic extract of Astragalus membranaceus.

Analogously to what has just been described from a morphological point of view, the expression levels of certain biochemical parameters, which are known due to their alteration in the course of damage, was also improved in rats that were administered the hydroalcoholic extract of Astragalus membranaceus at a dose of 300 mg/kg p.o. once daily simultaneously with oxaliplatin. At peripheral nerve level, the immunohistochemical evaluation has allowed us to use to highlight that the treatment in question was capable of increasing neuofilament expression levels (NF200) that were reduced due to the chemotherapeutic drug. A normalisation of the increased expression in the nuclei of ganglion neurons induced by chemotherapy has also been highlighted for transcription factor ATF3 (activating transcription factor 3), which is subject to nuclear dislocation in the course of damage.

Since in relation to the above-mentioned biochemical parameters, treatment with the hydroalcoholic extract of Astragalus membranaceus was responsible for a partial restoration of NF200 levels and for a decrease in the number of ATF3-positive nuclei, it can be stated that the hydroalcoholic extract of Astragalus membranaceus shows a neuroprotective effect in both SNP regions.

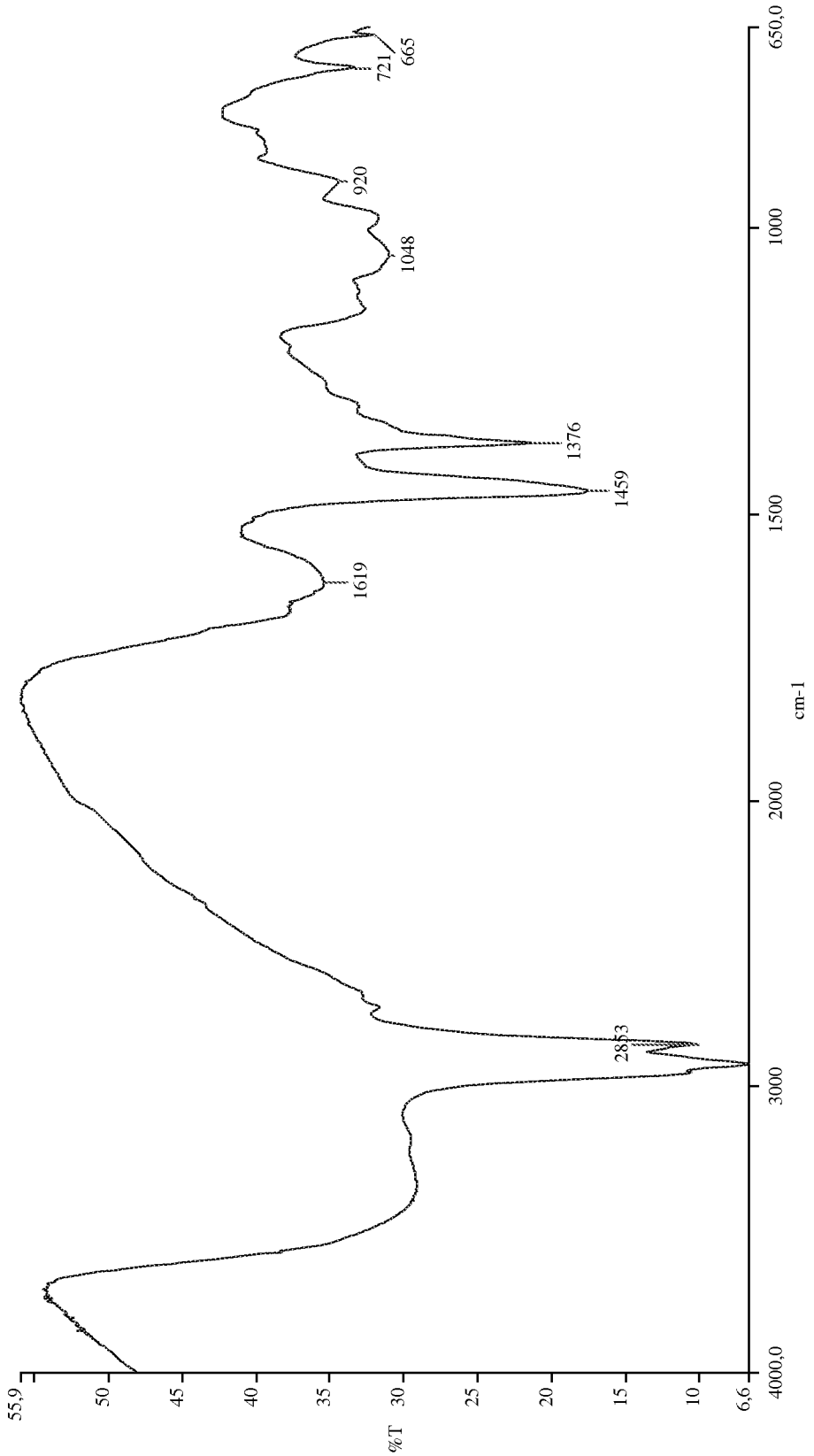
It has also been possible, at the level of the central nervous system (CNS), in particular in the spinal cord, using immunohistochemistry techniques in fluorescence microscopy, to highlight the significant role played by the hydroalcoholic extract of Astragalus membranaceus in normalising the density of the glial cells and, more particularly, of microglia and astrocytes that result numerically altered by treatment with oxaliplatin alone.

Claims

1. Hydroalcoholic or aqueous extracts of *Astragalus membranaceus* as antihyperalgesic and antiallodynic drugs.
2. Extracts according to claim 1, wherein said extracts are obtained by treating the dried, powdered root of *Astragalus membranaceus* with water or water/alcohol mixtures wherein the alcohol is between 0 and 80% (amount expressed in volume on the total amount of mixture).
3. Extracts according to claims 1 and 2, wherein said alcohol is ethyl alcohol.
4. Extracts according to claim 3, wherein ethanol is present in the amount of 70% (expressed in volume on the total amount of mixture).
5. Extracts according to claims 1 - 4, wherein the extraction is carried out at room temperature by maceration and under stirring for a few days in order to obtain an exhaustive extraction of the phytocomplex, possibly by renewing the solvent employed one or more times.
6. Extracts according to claims 1 to 4, wherein the extraction is carried out by percolation of the hydroalcoholic solutions or by decoction.
7. Process for preparing the extracts according to claims 1 - 5, wherein:
   - an amount of finely pulverised, dried root of *Astragalus membranaceus* is treated with an ethanol/water solution extracting under stirring by prolonged maceration;
   - the solvent is changed and the extraction continued until the exhaustive extraction of the phytocomplex;
   - the resulting solutions are pooled and the ethanol is evaporated with a rotary evaporator at low pressure;
   - the aqueous solution is lyophilized and the resulting powder is collected.
8. Pharmaceutical formulations for treating neuropathic pain, comprising extracts according to claims 1 - 6, in a form suitable for oral administration.
9. Formulations according to claim 8, wherein said neuropathic pain is of the post-herpetic pain, phantom limb pain, pain in peripheral neuropathies present in diabetes or in AIDS, pain in the so-called complex regional syndromes or reflex sympathetic dystrophies, and pain from lesions of the central nervous system.

10. Formulations according to claim 9, wherein said pain is neuropathic pain induced by chemotherapeutic drugs.

Fig. 1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2000
1500
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650,0
cm-1
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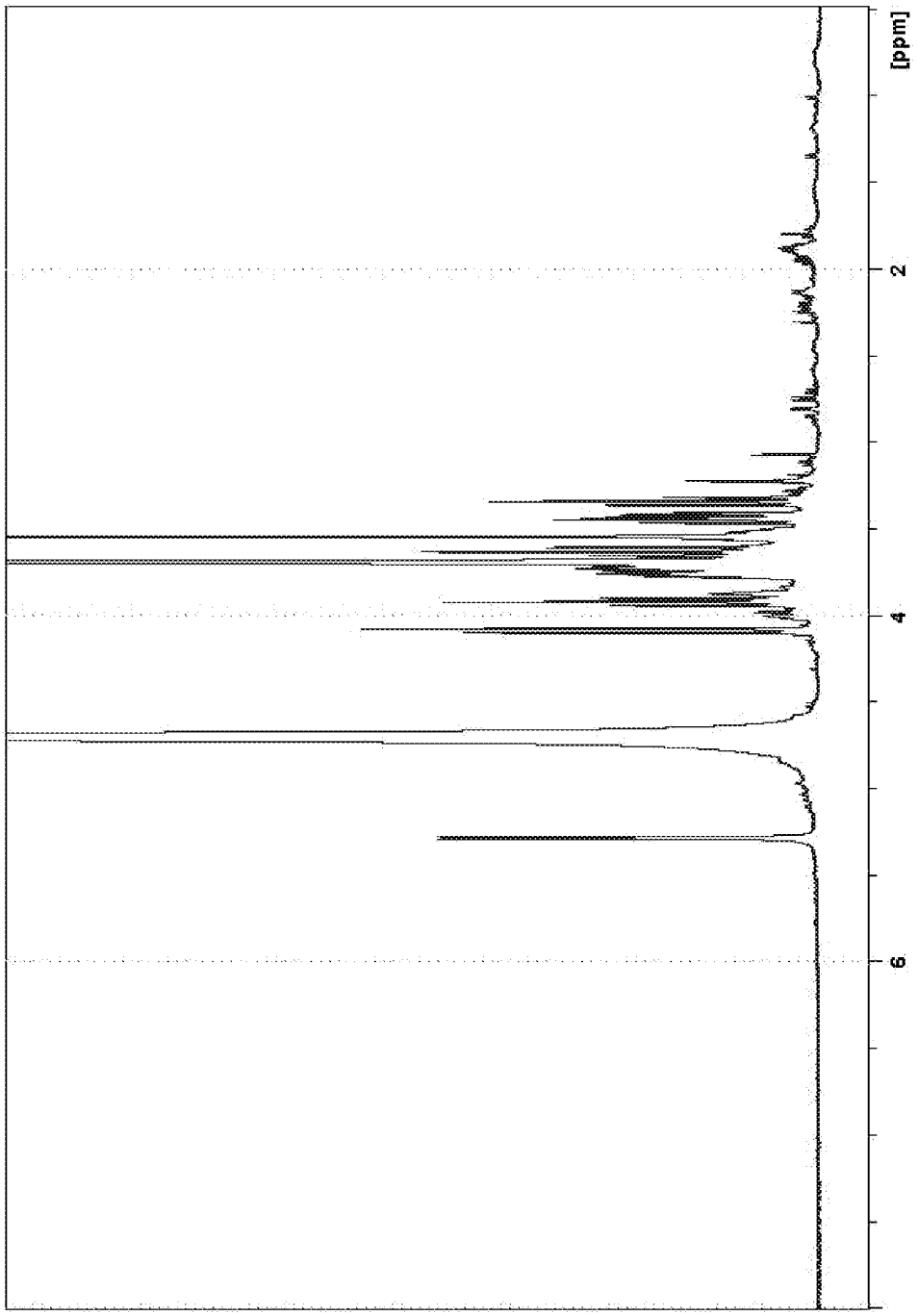
1619
1459
1376
1048
920
721
665

Fig. 2



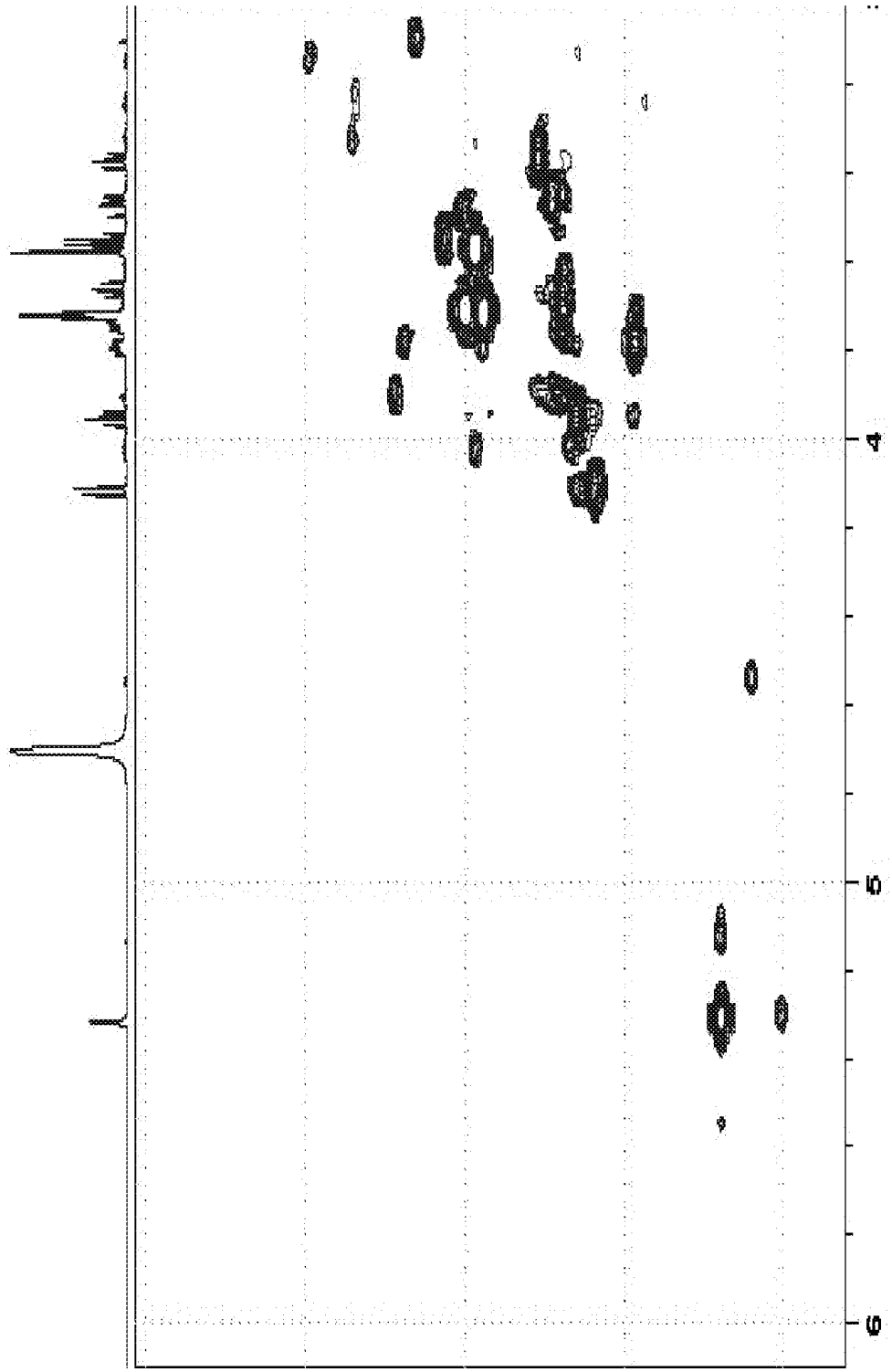


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/053914

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/481 A61P29/00
ADD.

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 A61P

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 practicable, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHIZUO TODA ET AL: "Inhibitory effects of Ougi-keishi-gomotsu-to, a traditional herbal medicine, on lipid peroxidation and protein oxidative modification of mouse brain homogenate induced by copper", PHYTOTHERAPY RESEARCH, vol. 13, no. 3, 1 May 1999 (1999-05-01), pages 251-253, XP55031525, ISSN: 0951-418X, DOI: 10.1002/(SICI)1099-1573(199905)13:3<251::AID-PTR428>3.0.CO;2-O	1,2,6, 8-10
Y	the whole document	1-10
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2013	05/08/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Thalmair-De Meyere

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/053914

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TODA S ET AL: "Inhibitory effects of Astragali Radix, crude drug in oriental medicines on lipid peroxidation and protein oxidative modification of mouse brain homogenate by copper", PHYTOTHERAPY RESEARCH, JOHN WILEY & SONS LTD. CHICHESTER, GB, vol. 14, no. 4, 1 June 2000 (2000-06-01), pages 294-296, XP002679152, ISSN: 0951-418X, DOI: 10.1002/1099-1573(200006)14:4<294::AID-PTR 627>3.0.CO;2-6 [retrieved on 2000-06-08]	1,2,6, 8-10
Y	the whole document	1-10
X	DATABASE TCM [Online] WIPO; 31 May 2006 (2006-05-31), Gao Pu: "Oral Chinese medicine preparation of polysaccharide glycoside and its preparation method / A new medicine composition used for treating diabetes and its complication and its preparation method", XP002704966, Database accession no. CN-20041091107-A	1-3,6, 8-10
Y	abstract & CN 1 778 311 A (GAO PU [CN] GAO PU BEIJING PUHUITIANYUE BI [CN]) 31 May 2006 (2006-05-31)	1-10
X	DATABASE TCM [Online] WIPO; 2 January 2008 (2008-01-02), Sima Lei: "A compound Chinese medicine for treating neuropathic pain", XP002704967, Database accession no. CN-200710142897-A	1,2,6, 8-10
Y	abstract & CN 101 095 789 A (LEI SIMA [CN] LEI SIMA) 2 January 2008 (2008-01-02)	1-10
Y	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; August 2012 (2012-08), LIU Y ET AL: "HPLC-MS3 identifying profiles of Astragalus membranaceus var. mongholicus for diabetic nephropathy and peripheral neuropathy", XP009171378, Database accession no. PREV201200599694 abstract	1-10

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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.
	& PLANTA MEDICA, vol. 78, no. 11, August 2012 (2012-08), pages 1255-1256, INTERNATIONAL CONGRESS ON NATURAL PRODUCTS RESEARCH ON GLOBAL CHANGE, NATURAL PRODUCTS AND HUMAN HEA; NEW YORK, NY, USA; JULY 28 -AUGUST 01, 2012 ISSN: 0032-0943(print) -----	
Y	LINDA L D ZHONG ET AL: "The efficacy of Chinese herbal medicine as an adjunctive therapy for colorectal cancer: A systematic review and meta-analysis", COMPLEMENTARY THERAPIES IN MEDICINE, vol. 20, no. 4, 4 March 2012 (2012-03-04), pages 240-252, XP028486272, ISSN: 0965-2299, DOI: 10.1016/J.CTIM.2012.02.004 [retrieved on 2012-02-15] the whole document -----	1-10