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21. **EXPEDIENTE No.** 07-28598

54. **TÍTULO** COMPOSICION DE PROTEINA ARABINOGALACTAN (AGP) PURIFICADA

51. **CLASIFICACIÓN INTERNACIONAL** A61K 35/78

71. **SOLICITANTE** LUPIN LIMITED

DOMICILIO Maharastra, India

74. **APODERADO** Dilia Rodriguez D'Alessio

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ENTRADA EN FASE NACIONAL

1) SOLICITUD DE:

☒ Patente de invención ☐ Patente de Modelo de Utilidad

☐ Capitulo I. ☒ Capitulo II.

2) SOLICITANTE (71)	**Nombre: LUPIN LIMITED** **Dirección: 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharastra, India** **Nacionalidad o domicilio: Maharastra, India** **Lugar de constitución: India**	**IDENTIFICACIÓN** **C.C.** ☐ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **Cual** _____ **NÚMERO** _____
	Teléfono: Fax: **E. Mail:**	
3) REPRESENTANTE O APODERADO	Nombre: DILIA MARÍA RODRÍGUEZ D´ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 6 Bogotá D.C. Teléfono: 6181088 Fax: 6350824 E. Mail: info@clarkemodet.com.co	**IDENTIFICACIÓN** **C.C.** ☒ **NIT:** ☐ **C.E.** ☐ **OTRO** ☐ **NÚMERO:** 41.654.882 **TP:** 20824 CSJ
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6)Título (54) COMPOSICIÓN DE PROTEÍNA ARABINOGALACTÁN (AGP)PURIFICADA

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Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta pagina

10) ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Comprobante de pago por reivindicación de prioridad
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- [x] Poderes, si fuera el caso
- [ ] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [x] Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- [x] Copia del examen preliminar efectuado por la IPEA
- [ ] De ser el caso, copia del contrato de acceso
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- [ ] De ser el caso, certificado de depósito del material biológico
- [ ] Arte final 12 x 12 y 6 x 6 cm
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- [x] Otros:
  1. Reporte Internacional de búsqueda
  2. Notificación de la recepción de la solicitud del IPEA
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  4. Opinión escrita

11) FIGURA CARACTERÍSTICA:

12) Solicitud concesión de la patente,

NOMBRE: DILIA RODRÍGUEZ D'ALEMAN

FIRMA:

C.C. 41.654.882 de Bogotá TP 20824 CSJ

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(74) Agents: SUBRAMANIAM, Hariharan et al.; Subramaniam, Nataraj & Associates, E-556, Greater Kailash-II, New Delhi 110 048 (IN).

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(54) Title: A PURIFIED ARABINOGALACTAN-PROTEIN (AGP) COMPOSITION

(57) Abstract: A purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant is described. Also described is a purified Arabinogalactan-Protein (AGP) composition isolated from the leaves and/or stems of *Argemone mexicana* plant, which has one or more of the following effects: immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, or IL-10 induction; keratinocyte proliferation inhibition, keratolytic activity and inhibitory activity in Mouse Ear Swelling test (MEST).

WO 2006/025068 A1

A PURIFIED ARABINOGALACTAN-PROTEIN (AGP) COMPOSITION

FIELD OF THE INVENTION

The present invention relates to a purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant.

The present invention also relates to a purified Arabinogalactan-Protein (AGP) composition isolated from the leaves and/or stems of *Argemone mexicana* plant, which has one or more of the following effects: immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, or IL-10 induction; keratinocyte proliferation inhibition, keratolytic activity and inhibitory activity in Mouse Ear Swelling test (MEST).

DESCRIPTION OF THE ABBREVIATIONS/NOTATIONS USED HEREIN

Arabinogalactan-Protein	: AGP
High Molecular Weight Arabinogalactan-Protein	: AGP - HM
Low Molecular Weight Arabinogalactan-Protein	: AGP – LM

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1. is a chart summarizing the selective process for isolating purified AGP composition.

Fig. 2: shows the proposed structure of AGP composition.

Fig. 3: shows the effect of AGP composition on IL-10 Induction.

Fig. 4: shows the effect of AGP composition on Percent IL-2 Inhibition.

Fig. 5: shows the effect of AGP composition on Percent IFNγ Inhibition.

Fig. 6: shows the effect of AGP composition on Inhibition of GMCSF.

Fig. 7: shows the effect of AGP composition on Inhibition of human TNFα.

Fig. 8: shows effect of AGP composition on NGF Induced Human Keratinocyte Proliferation.

Fig. 9: shows effect of AGP composition on NGF Induced Human Keratinocyte Proliferation.

Fig. 10: shows effect of AGP composition on Skin Thickness in PPD challenged guinea pigs.

Fig. 11: shows effect of AGP Composition on Inhibition of Epidermal Thickness Induced by TPA (12-O-tetradecanoylphorbol-13-acetate) in Balb/c mice.

Fig. 12: shows the effect of AGP Composition on DNFB Induced Mice Ear Swelling Test in Female C 57/BL 6 Mice.

BACKGROUND OF THE INVENTION

Psoriasis is a skin disorder characterized by inflammatory and abnormal epidermal keratinocyte hyper-proliferation resulting in hyperplasia, thickening of the epidermis and the presence of red scale plaques. The chronic skin condition is recognized for its peculiar clinical symptoms, characterized by circumscribed red patches covered with white scales that result in itchy, flaky skin. Psoriasis is a very visible disease and frequently affects the face, scalp, trunk and limbs. The lesions in this chronic disease typically are subject to remission and excerbations.

Although, psoriasis manifests as a skin disorder, while not being bound to any theory, it is believed to be a disease of impaired or defective cell mediated immunity. Since the clinical appearance of psoriasis is largely caused by epidermal changes, the disease has traditionally been considered one of excessive keratinocyte proliferation and abnormal differentiation. Current evidence suggests that epidermal changes in psoriasis are caused by actions of T lymphocytes in skin lesions and that T lymphocytes induce or sustain the disease process. Psoriasis is portrayed as an autoimmune disease, where activated T-lymphocytes, producing multiple cytokines cause secondary epithelial abnormalities. Dysregulated lymphocytes produce cytokines that stimulate the proliferation of apoptosis-resistant keratinocytes. Psoriatic skin lesions are characterized by inflammation, with T cells and neutrophils infiltrating both the dermis and epidermis and excessive scaling related to epidermal hyperproliferation and aberrant keratinocyte differentiation [Reich K., Garbe C., Blaschke V., Maurer C., Middel P., Westhal G., Lippert U., and Neumann C., *J. Invest. Dermatol.*, 2001, 116, 319].

Autoimmune disorders are diseases caused by the body producing an immune response against its own tissues. The cause of autoimmune diseases is unknown, but it appears that there is an inherited predisposition in many cases in the development of an autoimmune disease.

In a few types of autoimmune disease (such as rheumatic fever), a bacteria or virus triggers an immune response, and the antibodies or T-cells attack normal cells because they have some part of their structure that resembles a part of the structure of the infecting germ.

Autoimmune disorders fall into two general types: those that damage many organs (systemic autoimmune diseases), and those where only a single organ or tissue is directly damaged by the autoimmune process (localized). Some of the most common types of autoimmune disorders are summarized in below:

Systemic Autoimmune Diseases	*Localized Autoimmune Diseases*
Rheumatoid arthritis (joints; less commonly lung, skin)	Type 1 Diabetes Mellitus (pancreas islets)
Lupus [Systemic Lupus Erythematosus] (skin, joints, kidneys, heart, brain, red blood cells, other)	Hashimoto's thyroiditis, Graves' disease (thyroid)
Scleroderma (skin, intestine, less commonly lung)	Celiac disease, Crohn's disease, Ulcerative colitis (GI tract)
Sjogren's syndrome (salivary glands, tear glands, joints)	Multiple sclerosis*, Guillain-Barre syndrome (brain)
Goodpasture's syndrome (lungs, kidneys)	Addison's disease (adrenal)
Wegener's granulomatosis (sinuses, lungs, kidneys)	Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis (liver)

The inflammatory process involves a series of events that can be elicited by numerous stimuli (e.g. infectious agents, ischemia, antigen-antibody interactions, and thermal or other physical injury). Each type of stimulus provokes a characteristic pattern of response that represents a relatively minor variation on a theme. At a macroscopic level, the response usually is accompanied by the familiar clinical signs of erythema, edema, tenderness and pain.

The symptoms observed in psoriatic patients include hyperplasia and abnormal cornification of epidermal cells ascribed to the excess turnover of the cells by hyper metabolism, asthenia of inflammatory response in the epidermal layer, vasodilation and leukocyte migration and infiltration into the epidermal cell layers. However, it is now recognized that epidermal hyperplasia is a reaction to the activation of immune system in focal skin regions, which in turn, is mediated by CD8+ and CD4+ T lymphocytes that accumulate in the diseased skin. Indeed, psoriasis is now recognized as the most

prevalent T cell-mediated inflammatory disease of humans. The symptoms in psoriasis thus appear to be overly rapid growth of keratinocytes and shedding of scales from the skin surface. Within psoriatic lesions, the keratinocyte cell cycle time is reduced approximately 8 fold (36 vs. 311 hours in normal skin) and the number of dividing cell is doubled, resulting in a hyperplastic epidermis. Drug therapy is directed at slowing down this process.

It was found that PUVA therapy depleted lymphocytes in concert with disease improvements. These data are consistent with a role for T cells in pathogenesis. Cyclosporine, a known immunosuppressant was found to have dramatic effects on disease activity. Since cyclosporine has a major inhibitory effect on T cell activation, arguments began to be made that psoriasis was fundamentally an inflammatory disease.

T-lymphocytes must infiltrate the dermis and then adhere to keratinocytes to produce psoriatic plaque. Hence molecular regulating T cell adhesion and trafficking become tenable therapeutic targets and its role in pathophysiology is of considerable importance. Intravascular adhesion events can be inhibited by blocking chemokine triggering or blocking integrin binding (LFA-1 to ICAM-1). Integrin blockade or reduction of its surface expression could be an important event for lymphocytes trafficking which aid in anti-psoriatic therapy.

Immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, or IL-10 induction; keratinocyte proliferation inhibition, keratolytic activity and inhibitory activity in MEST are known to be involved in anti-psoriatic activity.

The number of different and sometimes toxic treatments employed for amelioration of psoriasis is testimony to the resistant nature of this disease. As the majority (90%) of psoriasis patients have limited forms of the disease, topical treatments that include dithranol, tar preparations, corticosteroids and the recently introduced vitamin D3 analogues (calcipotriol, calcitriol) can be used. A minority (10%) of psoriasis patients have a more serious condition, for which a number of systemic therapeutic modalities are available. Specific systemic therapies include UVB, PUVA, methotrexate, vitamin A derivatives (acitretin) and immuno-suppressants such as cyclosporin A. The effectiveness of cyclosporin and FK-506 for treating psoriasis provides support for the T cell hypothesis as the prime cause of the disease.

The topical use of corticosteroids reduces the symptoms of psoriasis. However their administration for a long period of time, which is necessary in such treatment causes tachyphylaxis so that either the dose has to be increased or stronger drugs have to be used leading to atrophy and achromasia or loss of pigmentation of peripheral normal skin, when it is topically applied on psoriatic lesion [*British National Formulary* (BNF), March 2001, No. 41].

Use of phototherapy (irradiation with ultraviolet radiation) or photochemotherapy, which consists of external or internal administration of psoralens and application of long wave ultraviolet rays to the affected part, is associated with disadvantages like the possibility of accelerated aging or pigmentation of the skin and of inducing carcinogenesis [*British National Formulary* (BNF), March 2001, No. 41].

External use of coal tar, even though is associated with fewer side effects when compared with steroids, is, however, messy and the drawbacks include strong odour, staining of skin etc. Occasionally it may cause stimulant dermatitis.

Methotrexate, even though it is a drug of choice for treating psoriatic conditions, needs to be closely monitored because it can cause liver damage and/or decrease the production of oxygen carrying red blood cells, infection-fighting white blood cells and clot-enhancing platelets. The long-term use of psoralens and methotrexate significantly increase the risk of squamous cell carcinoma in patients with psoriasis [Stern R. S., and Laird N., *Cancer*, 1994, 73, 2759].

The retinoids such as etretinate are taken internally by patients suffering from intractable psoriasis; however it is teratogenic and likely to accumulate in the body for a longer period of time and hence it is contraindicated in case of pregnancy [Stern R. S., and Laird N., *Cancer*, 1994, 73, 2759].

Use of macrocyclic immunosuppressive agents such as Cyclosporine, Tacrolimus and Ascomycin may impair kidney function or cause hypertension. Possible side effects of hydroxyurea include anemia and a decrease in white blood cells and platelets.

Calcipotriol, a synthetic vitamin D3 analogue has become one of the widely prescribed treatments for psoriasis. However, it causes significantly more skin irritation than potent topical corticosteroids. The common adverse effects include lesional or perilesional irritation, facial or scalp irritation, or excerbation of psoriasis [Ashcroft D. M., Wan Po A. L., Williams H.C. and Griffiths C.E.M., *BMJ*, 2000, 320, 963].

Current biotechnology approaches to psoriasis treatment relate to a direct pharmaceutical-mediated attack, either on cell proliferation or on the immune component of the disease. Immunosuppressive immunobiologicals such as Clenoliximab, MEDI-507, ICM3, IDEC-114, SMART Anti-CD3, Zenapax, Amavive, Hul 134, Xanelim, HuMaxCD4, IC747, IDEC-114 IDEC-131, Nuvion, DAB389IL-2, ONTAK and Etarnercept, known to block immune responses at various stages are currently under different phases of clinical trials.

None of the abovementioned treatments are, however, universally safe and effective. The magnitude of the impact of psoriasis is similar to that of other diseases like depression, hypertension and congestive heart failure. The cost of treating the disease averages 800 USD per patient per year in the United States, and the disease can cause significant loss in productivity [Feldman S.R., *American Academy of Dermatology*, August 2000].

Further, the disease owing to its sporadic course, gives variable response to treatments, which may also have adverse effects. Hence, it is a difficult disease to cure. The devastating nature of psoriasis is emphasized by the extent of the side effects that disease sufferers are willing to endure to attain a remission to a disease that they know will recur sooner or later.

In addition, apart from the clinical manifestations and inconvenience, the psychological impact of the disease on the patient's life is tremendous. Psoriasis is a complex condition affecting all aspects of emotion and physical debilitation for the patient and, substantially reduces the quality of life for millions of people all over the world. Moreover, as it is often clearly visible, affected individuals suffer marked distress, embarrassment and discomfort [Fortune D. G., Richards H. L., Main C. J., and Griffiths C. E. M., *J. Am. Acad. Dermatol.*, 1998, 39,196].

A composition derived from a plant source, which provides a safe, well-tolerated and effective treatment of psoriasis and which moreover, overcomes the shortcomings and limitations of the current treatments has been disclosed in our U.S. Patent Publication No. 2003/0194456 A1.

U.S. Patent Publication No. 2003/0194456 A1 discloses useful *in vitro* and *in vivo* immunological and pharmacological activities of a medication/composition comprising an extract obtained from the leaves and/or stems of the plant, *Argemone mexicana*, optionally in combination with an extract obtained from the fruits of the plant, *Cuminum cyminum* for the treatment and prophylaxis of psoriasis and other

disorders. The extract, which can be an aqueous, ethanolic or aqueous-ethanolic extract, apart from exhibiting useful immunological and pharmacological activities provides significant reduction in the rate of Psoriasis Area and Severity Index (PASI) score with better tolerability within the range of normal permissible limits. In proof of concept studies conducted on patients having chronic plaque type psoriasis, a composition comprising the abovementioned extract when administered orally was found to result in reduction of the PASI score from 6.33 ± 2.84 to 0.90 ± 1.27, with a disease free state observed in some patients after 8 weeks of treatment.

U.S. Patent Publication No. 2003/0194456 A1 further reports the acute toxicity (LD_{50}) of the extract obtained from the leaves and/or stems of the plant, *Argemone mexicana*, as evaluated in mice and rats through oral and i.v. routes of administration to be >1000mg/kg body weight of the animal with 50% mortality.

It might be mentioned herein that the *Argemone mexicana* plant is composed of various compounds, which include *inter alia:*

i) Alkaloids such as protopine, protopine nitrate, berberine, berberine nitrate, cryptopine, allocryptopine, coptisine, sanguinarine, dihydrosanguinarine, norsanguinarine, 6-acetonyl dihydrosanguinarine, dihydrochelerythrine, chelerythrine, norchelerythrine, 6-acetonyl dihydrochelerythrine, (-) cheilanthifolin, (-)-β-scoulerine methohydroxide, (-)-α- stylopine (-)-α and . β- stylopine methohydroxides, (-)-cheilanthifolin, 6-acetonyl dihydrosanguinarine, (-)-α-tetrahydropalmatine methohydroxide, reticuline, thalifoline, muramine, argemonine, norargeminine, argemexicaine A, argemexicaine B, N-demethyloxysanguinarine; (+)-1,2,3,4-tetrahydro-1-(2-hydroxymethyl-3,4-dimethoxyphenylmethyl)-6,7-methylenedioxy-isoquinoline, helleritrine, and oxyhydrastinine;

ii) Flavonoids, such as isorhmanetin, isorhamnetin-3-glucoside; isorhamnetin-3-O-glucoside, isorhamnetin-3,7-diglucoside; 3-methoxy quercetin, quercetin 5, 3',4' trimethyl ether; luteolin, argemexitin and eriodictyol;

iii) Fatty acids, such as palmitic, stearic, arachidic, oleic, linoleic, lauric, behenic, lignoceric, hexadecenoic, ricinoleic, 11-oxo-triacontanoic and 11-hydroxy triacontanoic;

iv) Amino acids, such as histidine, lysine, glutamic acid, glycine, alanine, leucine, valine, phenyl alanine, tyrosine, threonine, arginine, serine, asparagine,

cysteine, methionine, tryptophan, hydroxyproline, proline, L-glutamine, hydroxyproline, β-alanine, and aspartic acid;

v) Carbohydrates, such as glucose and fructose and glycosides;

vi) Organic acids, such as succinic, citric, tartaric, maleic, and malic; and

(vii) Other compounds like ceryl alcohol, β- sitosterol, potassium nitrate, calcium phosphate and calcium sulphate.

It has been found that the extract obtained from the leaves and/or stem of *Argemone mexicana* plant using the process for extraction e.g., maceration and percolation, using water, ethanol and mixtures thereof disclosed in U.S. Patent Application Publication No. 2003/0194456 A1 contains substantially all of the above-mentioned compounds. In other words, the extract is composed of all the compounds present in the parts of the plant used for extraction i. e., it is composed of a mixture of alkaloids, flavonoids, fatty acids, organic acids, amino acids, sugars and salts.

The extracts, thus obtained as per the process described in US Patent Application Publication No. 2003/0194456 A1 were found to exhibit *in vitro and in vivo* immunological and pharmacological activities e.g., immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFNγ inhibition, and IL-10 induction; keratinocyte proliferation inhibition, keratolytic activity, endothelial cell proliferation inhibition, inhibition of cell adhesion molecule expression such as ICAM-1, MEST inhibition, and enzymes inhibition such as p60src Tyrosine kinase, which are known to be involved in anti-psoriatic activity .

Furthermore, U.S. Patent Application Publication No. 2003/0194456 A1 teaches that the abovementioned extracts of the leaves and/or stem of *Argemone mexicana* plant could be fractionated using alcoholic solvents such as n-butanol and methanol and the fractions obtained thereof also exhibit *in vitro and in vivo* immunological and pharmacological activities including anti-psoriatic activity .

The fractionation procedure of the aqueous extracts of the leaves and/or stem of *Argemone mexicana* plant, described in Patent Application Publication No. 2003/0194456 A1 was achieved through a multi-step, liquid-liquid partition chromatography, precipitation and drying of extracts and provides fractions containing substantially different classes of compounds as major components.

For instance, as per the method described in U.S. Patent Application Publication No. 2003/0194456 A1 an n-butanol soluble fraction was prepared by adding n-butanol

to the aqueous extract of the leaves and/or stem of *Argemone mexicana* plant, separation of the n-butanol layer from the aqueous phase, followed by washing of the n-butanol layer with water and evaporation of the solvent under reduced pressure to give the n-butanol-soluble fraction as a viscous mass. The aqueous layer, was mixed with methanol, wherein precipitation of a solid mass was effected. The solid mass was separated, and the mass dissolved in water and lyophilized to give the methanol-insoluble fraction. The filtrate obtained after separation of the methanol-insoluble precipitate from the methanol-water mixture on evaporation gave the methanol-soluble fraction as a solid mass.

Typically, *Argemone mexicana* plant yielded about 3-4.5% of n-butanol-soluble fraction; 46-54% of methanol-soluble fraction, having a total base number between 290-340; and 24-30% of methanol-insoluble fraction, having a total base number between 350-380. As used herein base number is the quantity of acid that is required to neutralize all basic constituents present in 1 g of sample.

As mentioned hereinbefore, the three fractions differ substantially in the constitution of compounds contained therein. The n-butanol-soluble fraction was found to contain alkaloids, flavonoids and other low molecular weight compounds; the methanol-soluble fraction was found to contain amino acids, organic acid and salts; while the methanol-insoluble fraction was found to contain sugars, organic acids and salts.

Even though, extensive chemical investigations over the years on different parts of the Argemone mexicana plant have resulted in the isolation of a number of alkaloids, flavonoids, amino acids, organic acids, fatty acids etc., however, no systematic study has been conducted and reported for isolation and identification of other principles present in the plant.

One such principle, commonly found in the plant kingdom is Arabinogalactan-Proteins (AGP), which are essentially macromolecules of polysaccharides in which the carbohydrate is associated with or linked to proteins. AGP is composed mainly of arabinose and galactose residues. These occur in plants as polysaccharides in association with varying amounts of proteins, and generally contain a high proportion of carbohydrates with comparatively less proportion of proteins, usually less than 10 of proteins, although, AGPs having higher contents of proteins are also known. AGPs are widely distributed in most of the higher plants such as *Echinacea purpurea, Nicotiana alata, Vitis vinifera, Diospyros kaki, Gladiolus gandavensis, Lolium multiflorum*

[Anderson, R.L., Clarke, A.E., Jermyn, M.A., Knox, R.B. and Stone, B.A., Australian *J. Plant Physiol.*, 1977, 4, 143-158], *Phaseolus vulgaris* [Hawes, G. B., Adams, G.A., *Phytochemistry*, 1972, 11, 1461-1465] and *Acacia arabica* [Classen, B., Witthohn, K., and Blaschek, W., *Carbohydrate Research*, , 2000, 327, 497-501].

AGPs possess adhesive and water holding properties and respond to wounds and infections in plants. These determine cellular identity and specific interactions. They also play a role in cell and tissue differentiation as well as in controlling somatic embryogenesis. They are also valued for various biological activities [*WO 01/00682 A1*, 2001]. There are two types of AGPs, viz. AGP I and AGP II. The latter i. e. AGP II contain a galactose core and are highly branched, contain usually less than 10% proteins and possess side chains highly substituted by arabinofuranosyl residues and sometimes other sugars like rhamnose, glucose, mannose etc. Presence of uronic acids and substituted derivatives are also reported.

As mentioned hereinbefore, even though, chemical investigations on all parts of *Argemone mexicana* have been conducted, however, no study has been directed towards isolation, characterization and understanding of the biological properties of AGPs present in certain parts of the plant.

SUMMARY OF THE INVENTION

The present invention provides a selective method for isolation of AGPs from an extract obtained from the leaves and/or stems of the plant, *Argemone mexicana* in purified form, which exhibits vastly superior anti-psoriatic activity and other useful immunological and pharmacological activities compared to the extract and fractions of the leaves and/or stems of the plant, *Argemone mexicana*, as disclosed in US Patent Application Publication No. 2003/0194456 A1. In particular, the vastly superior anti-psoriatic activity exhibited by the purified AGPs obtained by the selective method is found to be highly useful in preparation of a pharmaceutical composition comprising the same and thereby providing a safe, effective and well-tolerated treatment and form the basis of the present invention.

Therefore, an aspect of the present invention is to provide a selective method for isolation of purified Arabinogalactan-Protein (AGP) composition in a highly pure form from the leaves and/or stems of *Argemone mexicana* plant.

In another aspect, the present invention provides a purified Arabinogalactan-Protein (AGP) composition, having an average molecular weight range between 10 KD to 150 KD, isolated from the leaves and/or stems of *Argemone mexicana* plant.

Another aspect of the present invention is to provide an anti-psoriatic composition and treatment for psoriasis, obtained from the leaves and/or stems of *Argemone mexicana* plant, which not only is highly safe, effective and well-tolerated but moreover, overcomes the shortcomings and limitations of the current therapeutic regimen.

Another aspect of the present invention is to provide a purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant which exhibits useful immunological and pharmacological properties such as one or more of immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, or IL-10 induction;, keratinocyte proliferation inhibition, keratolytic activity and MEST inhibition.

Yet another further aspect of the present invention is to provide a purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant for treatment or prophylaxis of one or more of disorders such as dermatitis; scleroderma; eczema; inflammatory disorders and other autoimmune diseases like psoriatic arthritis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, irritable bowel disease, ankylosing spondilitis, systemic lupus erythrometosus and Sjogren's syndrome; and/or allergies like asthma and chronic obstructive pulmonary disease.

Yet another aspect of the present invention is to provide a pharmaceutical composition that can be used for the treatment and/or prophylaxis of the aforementioned diseases and conditions compriaing the purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all technical and scientific terms have the same meaning as commonly understand by one of ordinary skill in the art to which this invention below.

As used herein the phrase "purified Arabinogalactan-Protein (AGP) composition" means a composition consisting essentially of only arabinogalactan-proteins which may include trace amounts of other components obtained from extraction of the stem and/or leaves of *Argemone mexicana* plant according to this

invention. The purified AGP composition is distinguished from a pharmaceutical composition comprising AGP composition because the purified AGP composition does not include any pharmaceutically acceptable excipients.

In our U.S. Patent Application Publication No. 2003/0194456 A1 a process was disclosed for obtaining an extract from the leaves and/or stems of the *Argemone mexicana* plant and fractionation of the extract with n- butanol and methanol to give the respective n- butanol-soluble, methanol- soluble and methanol- insoluble fractions.

The method for preparation of the various fractions of the stem and/or leaves of *the Argemone mexicana* plant as disclosed in US Patent Application Publication No. 2003/0194456 A1 comprises the steps of:

i) preparation of an extract of the stem and/or leaves of *the Argemone mexicana* plant, through a number of extraction procedures, however, preferably through a multi-step, successive maceration and percolation using solvents such as water, ethanol or mixtures thereof at room temperature to obtain the corresponding extracts, which are constituted of alkaloids, flavonoids, amino acids, organic acids, sugars and salts. These extracts could be used as such or were subjected to lyophilization to give a lyophilized mass, both of which were used for fractionation;

ii) partitioning of the extract as obtained or a solution of the lyophilized mass in water as obtained in step i) with n-butanol, and separation of the n-butanol layer from the aqueous phase, followed by washing of n-butanol layer with water and evaporation of the solvent under reduced pressure to give a viscous mass of the n-butanol-soluble fraction, which is constituted primarily of alkaloids, flavanoids and other low molecular weight compounds;

iii) mixing and agitation of the aqueous layer from step ii) with approximately six to seven times its volume of methanol and filtration/centrifugation of the precipitated solids and drying to give the methanol-insoluble fraction, which is constituted primarily of polysaccharides, organic acids and salts. This material was dissolved in water and lyophilized to give a lyophilized powder; and

iv) Concentration of the filtrate i.e. aqueous methanolic solution from step iii) under reduced pressure to give a solid mass of the methanol-soluble fraction, which is constituted primarily of amino acids, organic acids and inorganic salts.

The methanol-insoluble fraction prepared according to the method disclosed in US Patent Application Publication No. 2003/0194456 A1 was isolated in a yield of about 33%. While there is mention in the specification that the fraction is constituted

of sugars, organic acids and salts, however, these constituents were not characterized at that time.

Present investigations reveal that the methanol-insoluble fraction obtained as per the method disclosed in US Patent Application Publication No. 2003/0194456 A1 is constituted of approximately 3% by weight of AGPs, (with respect to the aqueous extract), the remaining constituents being organic acids, amino acids and inorganic salts i. e. the methanol-insoluble fraction obtained according to the method disclosed in U.S. Patent Application Publication No. 2003/0194456 A1 is constituted of AGPs in mixture with other components. The methanol-insoluble fraction contains AGPs, albeit in low concentration and in a highly impure form.

The present inventors have found that AGPs can be isolated in a purified form in a yield of approximately 0.06% yield with respect to the stems and/or leaves of Argemone mexicana or approximately 1% with respect to the lyophilized aqueous extract prepared therefrom through a highly selective method and that the isolated AGPs exhibit vastly superior anti-psoriatic activity over the ones obtained through the method disclosed in US Patent Application Publication No. 2003/0194456 A1.

The selective method for isolation of the AGPs in purified form from the leaves and/or stems of the *Argemone mexicana* plant comprises the steps of:

a) extraction of 1 wt part of the leaves and/or stems of *Argemone mexicana* plant with 1 to 10 wt part of water, a C_1-C_3 alcohol or a mixture thereof to obtain an aqueous extract which can be but does not have to be partially or completely concentrated or lyopholized;

b) removal of the basic and acidic components from the aqueous extract, partially concentrated extract, an aqueous solution of the completely concentrated extract or lypholized extract obtained in step a) by subjecting the partially concentrated extract or the aqueous solution of the concentrated extract to ion exchange chromatography to obtain a neutral aqueous extract;

c) fractionation of the neutral aqueous extract obtained in step b) with n-butanol to give a n-butanol-soluble fraction;

d) mixing and agitation of the aqueous washes from step c) with methanol or ethanol and isolation of the precipitated solids to obtain the methanol or ethanol-insoluble fraction; and

e) subjecting the methanol or ethanol-insoluble fraction obtained in step d) to gel chromatography and size exclusion chromatography in succession to obtain purified Arabinogalactan-Protein (AGP).

The selective method for isolation of the purified AGP is summarized in Fig. 1.

For extraction, leaves, stems or both of the *Argemone mexicana* plant can be used. Preferably fresh leaves, stems or both are used, and these are ground to a coarse or fine paste prior to extraction.

In an embodiment of the invention , one wt part of the fresh ground leaves and/or stems of *Argemone mexicana* are extracted with 1 to 10 wt parts of water, C_{1-3} alcohol, or a mixture thereof 2 to 4 times and the combined extracts percolated for 2 to 20 hours at a temperature of between 20EC to 45EC.

In another embodiment, one wt part of the fresh ground leaves and/or stems of *Argemone mexicana* are extracted with 1 to 3 wt parts of water, C_{1-3} alcohol, or a mixture thereof 2 to 4 times and the combined extracts percolated for 2 to 16 hours at a temperature of between 20EC to 45EC.

In another embodiment, one wt part of the fresh ground leaves and/or stems of *Argemone mexicana* are extracted with 1 to 1.5 wt part of water, a C_{1-3} alcohol, or a mixture of water and the C_{1-3} alcohol thereof, 2 to 4 times and the combined extracts percolated for 16 hours at room temperature.

The C_1-C_3 alcohol is selected from methanol, ethanol, 1-propanol and 2-propanol, preferably ethanol.

After percolation, the extract is filtered or centrifuged and the filtrate can be partially concentrated to a certain volume of the original volume of the extract or can be concentrated to dryness or can be lyophilized.

When the extract is partially concentrated it is concentrated to a volume of between $1/5^{th}$ to $1/10^{th}$ of the original volume of the extract. When the original extract is concentrated to dryness or lyophilized, the dried extract or the lyophilized powder can be redissolved in 12 to 50 times by wt of water to one part by wt of the dried/lyophilized mass prior to ion exchange chromatography.

In a preferred embodiment the lyophilized aqueous extract was dissolved in 12 times water prior to ion exchange chromatography.

Either the solution of the partially concentrated extract or the aqueous solution of the concentrated or lyophilized obtained on complete concentration of the original

extract is then subjected to sequential ion exchange chromatography over a cation exchange resin followed by chromatography over an anion exchange resin or in another embodiment ion exchange chromatography over an anion exchange resin followed by chromatography over a cation exchange resin to afford a neutral aqueous extract.

The cation and anion exchange resins are employed in proportions of 1 part to 50 parts by wt to the volume of the extract used.

Suitable cation exchange resins that can be employed include sulphonated polystyrene strong-acid cation exchangers and carboxylic acid-type weak acid cation exchangers. Suitable cation exchange resins include but are not limited to commercially available and commonly used sulphonated polystyrene strong-acid cation exchangers like AG 50W (Bio-Rad, USA), Amberlite IR-20 (Rohm and Haas, USA), Dowex 50W (Dow Chemical Co., USA), Duolite 225 (Dia-Prosim Ltd), Permutit RS (Permutit AG, Germany), and Permutite C50D (Philips and Pain-Vermorel, France); and carboxylic acid-type weak acid cation exchangers like Amberlite IRC-50 (Rohm and Haas, USA), Bio-Rex 70 (Bio-Rad, USA), Chelax 100 (Bio-Rad, USA), Duolite 436 (Dia-Prosim Ltd), Permutit C (Permutit AG, Germany), and Permutit H and H-70 (Permutit Co., USA).

Suitable anion exchange resins that can be employed include aliphatic amine-type weak base anion exchangers and strong base anion exchangers. The anion exchange resins include but are not limited to the commercially available and commonly used aliphatic amine-type weak base anion exchangers like Amberlites IR-45 and IRA-67 (Rohm and Haas, USA), Dowex 3 (Dow Chemical Co., USA), Permutit E (Permutit AG, Germany), Permutit A 240A (Philips and Pain-Vermorel, France); and strong base anion exchangers like AG 2x8 (Bio-Rad, USA), Amberlite IRA-400 (Rohm and Haas, USA), Dowex 2x8 (Dow Chemical Co., USA), Duolite 113 (Dia-Prosim Ltd), Permutit ESB (Permutit AG, Germany), and Permutite 330D (Philips and pain-Vermorel, France).

The neutral aqueous extract thus obtained can be lyophilized and the lyophilized mass can be partitioned between n-butanol and water for the next fractionation step. In the alternative, the neutral aqueous extract can be used as such for fractionation with n-butanol. For cost-effectiveness, it is preferable to use the solution of neutral fraction obtained after passing through cation and anion exchange resins as such for fractionation with n-butanol.

Typically, the abovementioned lyophilized mass of the neutral extract is partitioned in a mixture of water and n-butanol per 1 part of the lyophilized mass. The solution is allowed to stand and the n-butanol layer separated from the aqueous phase. The step is repeated 2 to 4 times and the combined aqueous layers are used for further fractionation with methanol or ethanol.

In an example of the invention 1 part by volume of the solution of neutral aqueous fraction obtained after passing through cation (Amberlite IR 120) and anion (Amberlite IRA 400) exchange resins is added to about 10 parts by volume of n-butanol. The phases are mixed, allowed to stand and the n-butanol layer separated from the aqueous phase. The step is repeated 2 to 4 times and the combined aqueous layers are used for further fractionation with methanol or ethanol.

The combined aqueous phase obtained in the above-mentioned step is mixed with methanol or ethanol and agitated to precipitate out the methanol/ethanol-insoluble fraction. Typically, methanol or ethanol is employed in proportions of 1 to 20 times volume per 1 volume of the aqueous extract.

The precipitated solid, which contains the AGP is isolated by conventional means, such as decantation, filtration, centrifugation, etc. and then dried to yield a brownish amorphous powder.

The solid AGP thus obtained, which contains AGP-HM and AGP-LM is further subjected to sequential gel chromatography and size exclusion chromatography to obtain purified AGP-HM and AGP-LM.

The gel chromatography is carried out using conventional techniques and polymeric adsorbents such as Amberlite adsorbents like XAD-2, XAD-4 or XAD-7, preferably XAD-7. The impure solid AGP is applied in a narrow band at the top of the requisite column and washed by the mobile phase, which is water. The fractions containing the AGP are collected.

The elute containing the impure AGP is further subjected to size exclusion chromatography using conventional techniques. Sephacryl (1:25 - 1:50) may be used to obtain purified AGP, AGP (HM) and AGP (LM) of the present invention. Commercially available Sephracyl S-100, S-200 HR and S-300 HR can be used, the preferred one being S-200 HR. The Sephracyl can be employed in a ratio of 1 to 2 parts by weight to 25 to 250 parts by volume of the solution of AGP obtained after gel chromatography.

In an embodiment, the crude AGP (100mg) is dissolved in water and loaded on sephacryl S-200 (25ml). The column is eluted at a rate of 0.5ml/minute and fifty fractions were collected. All fractions were monitored by HPLC-ELSD. Fractions 10 to 20 were combined on the basis of HPLC to yield AGP-HM and fractions 25 to 35 were also combined to yield AGP-LM, the components of AGP composition having an average molecular weight range between 10 KD to 150 KD.

Following size exclusion chromatography, the purified AGP of the present invention can be isolated from the aqueous solution through employment of conventional techniques such as evaporation, lyophilization, spray drying, freeze drying etc.

The purified AGP, thus obtained is found to show an average molecular weight range between 10 KD to 150KD. It is found to exhibit one or more of the following effects: immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, IL-10 induction, keratinocyte proliferation inhibition, keratolytic activity and MEST inhibition. These are known to be involved in inflammatory disorders, autoimmune diseases and allergies. These are also known to be involved in anti-psoriatic activity, dermatitis, scleroderma, eczema and scaly itchy patches. Inflammatory disorders and autoimmune diseases include psoriatic arthritis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, irritable bowel disease, ankylosing spondilitis, systemic lupus erythrometosus , Sjogren's syndrome. Types of psoriasis include plaque psoriasis, guttate psoriasis, pustular psoriasis and psoriasis of the nails. Allergies include asthma and chronic obstructive pulmonary disease. IL-10 induction is also useful in other chronic, recurrent and other skin ailments where cutaneous lymphocyte antigen or cutaneous leukocyte antigen is involved.

The purified Arabinogalactan-Protein (AGP) composition exhibits excellent antipsoriatic activity *in vitro* and *in vivo*, mediated via IL-10 induction.

The purified AGP of the present invention (which is water-soluble) obtained after size exclusion chromatography over, for example, sephacryl is constituted of several fractions of differing molecular weights. Such fractions have average molecular weights as high as between 115 to 150 KD and as low as between 10 to 15 KD. The AGPs possessing high molecular weights are termed as AGP-HM, while those of low molecular weights are termed as AGP-LM. A typical AGP composition is thus constituted of AGP-HMs and AGP-LMs in varying proportions. It should be

understood that the aforementioned AGP is formed through a biogenetic pathway, wherein the carbohydrates/monosaccharides are constantly seeking a protein for covalent bond formation, leading ultimately to low molecular weight (AGP-LMs) and high molecular weight (AGP-HMs) AGPs. Such a covalent bond formation is constantly propagated and whether in one particular method one ends up with an AGP-LM of average molecular weight less than 10 KD or an AGP-HM of average molecular weight greater than 150 KD would depend on several factors, which are to name a few the nature of the parts of the plant used i.e. whether fresh leaves or stems of the *Argemone mexicana* plant has been used for extraction, the harvesting conditions of the leaves i.e. whether harvested in the rainy season etc. Hence, it is possible that a purified AGP composition having an average molecular weight outside the range of 10 KD to 150 KD could be obtained employing the method of the present invention depending on the abovementioned factors. In view of the above, AGP composition with an average molecular weight outside the range of 10KD to 150KD would still be within the scope of the invention. Examples of such AGP compositions are those with a lower limit of an average molecular weight of 9 KD or AGP compositions with an upper limit of average molecular weight to 155 KD.

The bulk of the AGP obtained by the present method is constituted of carbohydrates, with a smaller proportion of proteins. The carbohydrate components of the AGP are Arabinose, Rhamnose, Methylated Uronic acid, Mannose, Galactose, Glucose and other unidentified sugars, which are linked to a protein component consisting of various amino acids, which are identified as Asparagine, Glutamine, Hydroxyproline, Serine, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Isoleucine, Leucine, Lysine, Phenylalanine etc., as would be evident from Tables-I and II of the specification.

It is noticed that not only is the glycosyl composition of both the AGP-HMs and AGP-LMs similar i. e. they show similarity in the ratio of monosaccharides and proteins present therein but the glycosyl linkage in both the cases are also similar.

Further, it is noted that the biological activity of both the two molecular weight components are comparable and do not show much variation. That is to say, a purified AGP-LM having a molecular weight of 10 KD exhibits one or more of immunosuppression; lymphoproliferation inhibition; cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, and IL-10 induction; keratinocyte proliferation inhibition,

keratolytic activity, MEST inhibition and most importantly anti-psoriasis activity which is comparable to those exhibited by a purified AGP-HM having a molecular weight of 150 KD.

The AGP composition of this invention is composed of AGPs (both AGP-HM and AGP-LM) which is substantially free of other components present in the extract of the plant, which are a mixture of alkaloids, flavonoids, organic acids, amino acids, sugars, polysaccharides, proteins, proteoglycans (excluding AGP) and salts. As used herein the term "substantially free" is intended to cover AGP compositions containing from about 0.0001% by weight to about 10% by weight of other components of the extract of the plant.

The n-Butanol fraction contains a mixture of alkaloids (such as protopine, protopine nitrate, berberine, berberine nitrate, cryptopine, allocryptopine, coptisine, sanguinarine, dihydrosanguinarine, norsanguinarine, 6-acetonyl dihydrosanguinarine, dihydrochelerythrine, chelerythrine, norchelerythrine, 6-acetonyl dihydrochelerythrine, (-) cheilanthifolin, (-)-β-scoulerine methohydroxide, (-)-α- stylopine methohydroxide, 6-acetonyl dihydrosanguinarine, (-)-α-tetrahydropalmatine methohydroxide, reticuline, thalifoline, muramine, argemonine, norargeminine, helleritrine, and oxyhydrastinine) , flavonoids (such as isorhmanetin, isorhamnetin-3-glucoside and isorhamnetin-3,7-diglucoside) and other low molecular weight compounds;

The Methanol-soluble fraction contains amino acids (such as histidine, lysine, glutamic acid, glycine, alanine, leucine, valine, phenylalanine, tyrosine, threonine, arginine, serine, asparagine, cysteine, methionine, tryptophan, hydroxyproline, proline and aspartic acid), sugars and some salts; and

The Methanol-insoluble fraction contains some organic acids (such as succinic, citric, tartaric and malic), monosaccharides, polysaccharides, proteoglycans (including AGP) and salts.

In an embodiment of the invention, the AGP compositions contain less than 1 % by weight of other components of the extract of the plant.

The purified AGP composition isolated by the process of this invention is found to be an excellent inducer for IL-10 in ConA activated human PBMCs. It produced about 371% induction at a concentration of 200 µg/ml, which is vastly superior to a value of about 171% exhibited by the methanol-insoluble fraction obtained through the

method disclosed in US Patent Application Publication No. 2003/0194456 A1 at the same concentration of 200 µg/ml.

More particularly, the isolated AGP composition is remarkable in that it exhibits IL-10 induction equal to or greater than that exhibited by the methanol-insoluble fraction obtained through the method disclosed in US Patent Application Publication No. 2003/0194456 A1 even at a very low concentration of 0.2 to 2.0 µg/ml.

A comparison of the effect of concentration of the AGP of the present invention on IL-10 Induction by ConA Induced Human PBMCs with that of the Methanol-Insoluble Fraction Prepared by the Method Described in US Patent Application No. 2003/0194456 A1 is summarized in Table-IA

TABLE IA

Comparison of the Effect of the AGP Composition of the Present Invention on IL-10 Induction by ConA Induced Human PBMCs with that of the Methanol-Insoluble Fraction Prepared by the Method Described in U.S. Patent Application No. 2003/0194456 A1.

Sr. No.	*Concentration of the AGP Composition of the Present Invention (µg/ml)*	*Average Percent Induction*	*Concentration of the Methanol-Insoluble Fraction obtained by the Method of US Application No. 2003/0194456 A1*	*Average Percent Induction (As given in Table-8 of US Application No. 2003/0194456 A1*
01	200.00	371.40	200.00	171.10
02	20.00	303.80	20.00	162.10
03	2.00	237.30	2.00	98.30
04	0.20	137.20	0.20	24.60
05	0.02	114.30	0.02	-4.00
06	0.002	106.00	0.002	-5.00
07	0.0002	102.60	0.0002	–

*The values are depicted in percent increase from basal with reference to control.

** The Average % Induction exhibited by the methanol-insoluble fraction obtained through the method disclosed in US Patent Application No. 2003/0194456 A1 was 171% at a concentration of 200 µg/ml.

Such vastly potent activity exhibited by the AGP obtained by the method described hereinbefore enables it to be administered at a substantially reduced concentration of 1/100th to 1/1000th of the dosage required to be administered using the extracts/fractions obtained by the method disclosed in U.S. Patent Application No.

2003/0194456 A1 thereby providing a cost-effective, efficient and well-tolerated treatment for psoriasis and other disorders.

The present invention provides pharmaceutical compositions that comprise an effective amount of the purified Arabinogalactan-Protein (AGP) composition, having an average molecular weight range between 10 KD to 150 KD, isolated from the leaves and/or stems of *Argemone mexicana* plant by the selective method enumerated hereinabove, in admixture with pharmaceutically acceptable excipients. The compositions of this invention are safe, effective and well-tolerated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve its desired purpose. The term an effective amount means that amount of AGP composition that will elicit the biological or medical response of a tissue, cell, system, animal, non-human mammal, or human mammal that is being sought. This is intended to refer to situations where there may be a slowing, interrupting, arresting or stopping of the progression of the diseases and/or conditions described herein, but does not necessarily indicate a total elimination of all disease and condition symptoms, but does include prophylactic treatment of diseases and/or conditions..

The AGP composition of the present invention can be formulated into a suitable dosage form in admixture with one or more pharmaceutically acceptable excipients such as carriers, diluents, fillers and the like. Suitable dosage forms are forms suitable for oral administration or topical application. Non-limiting examples of such dosage forms are liquids, dry powder or powdered concentrate, capsule, tablet, pellet, granules, gels, ointments, creams, emulsions, suspensions, dispersions, lotions, pills and the like.

Generally a typical pharmaceutical composition for oral administration comprises the AGP composition as active ingredient in an amount in the range between 50-5000 mg , more preferably 200 mg.

Generally a typical pharmaceutical composition for topical administration comprises the AGP composition as active ingredient in an amount in the range between 0.1-10% by weight , more preferably 2% by weight.

The prophylatic or therapeutic dose of the AGP composition or compositions containing AGP composition is from 50 mg and 5000 mg per day, preferably 200 mg dose per day. The dose may be administered as a single or divided dose.

However, the exact formulation, route of administration and dosage can be chosen by the patient or health care professional in view of the patient's condition and whether the patient is presently afflicted with the disease or condition or whether the treatment is prophylatic. A prophylatic dosage can be administered to a patient who is at risk for developing a disease or condition. The risk factors include but are not limited to genetic and environmental risk factors. It is preferred to administer the pharmaceutical composition at a dose that will produce the desired result without causing undue side effects. As used herein, the term "patient" means animal, non-human mammal, or human mammal.

Suitable dosage forms for oral administration and topical application comprising the AGP composition of the present invention in admixture with pharmaceutically acceptable carriers can be prepared.

Suitable forms of oral administration include tablets, capsules, powdered concentrate, syrups, elixirs or suspensions. Suitable forms of topical application include ointments, creams, lotions, oils or transdermal drug delivery systems.

Suitable pharmaceutically acceptable excipients include sugars such as lactose, sucrose, mannitol, sorbitol and xylitol; Starches such as corn starch, tapioca starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; Calcium phosphates such as dicalcium phosphate and tricalcium phosphate; Sodium sulphate; Calcium sulphate; Polyvinylpyrrolidone; Polyvinyl alcohol; Stearic acid; Vegetable oils such as peanut oil, cottonsead oil, sesame oil, olive oil and corn oil; Non-ionic, cationic and anionic surfactants; Ethylene glycol polymers; β-cyclodextrin; Fatty alcohols; Hydrolysed cereal solids; as well as other Non-toxic compatible fillers, Binders, Disintegrants, Buffers, Preservatives, Antioxidants, Lubricants, Flavouring agents etc.

The compositions of this invention are prepared according to conventional techniques known in the art.

The compositions are pharmaceutically acceptable meaning that they are suitable for use with humans and/or animals.

The AGP compositions and pharmaceutical compositions of this invention are useful in the treatment and/or prophylaxis of the diseases and conditions described herein.

The AGP composition of this invention or a pharmaceutical composition comprising AGP composition of this invention can be used in the treatment and/or prophylaxis of psoriasis skin ailments such as psoriasis including plaque psoriasis, guttate psoriasis, pustular psoriasis and psoriasis of the nails comprising administration to a mammal in need of such treatment or a mammal at risk for psoriasis skin ailments an effective amount of the AGP composition or a pharmaceutical composition comprising a therapeutically effective amount of the purified Arabinogalactan-Protein (AGP) composition.

The AGP composition or a pharmaceutical composition comprising the AGP composition can be used in the treatment or prophylaxis of inflammatory disorders; autoimmune diseases like psoriatic arthritis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, irritable bowel disease, ankylosing spondilitis, systemic lupus erythrometosus and Sjogren's syndrome; allergies like asthma and chronic obstructive pulmonary disease. The treatment or prophylaxis comprises administration to a mammal in need of such treatment or a mammal at risk for developing or experiencing an outbreak of one of these disorders or diseases an effective amount of the AGP composition or a pharmaceutical composition comprising a therapeutically effective amount of the purified Arabinogalactan-Protein (AGP) composition.

Compositions of this invention which cause immunosuppression, are also useful for patients who expect to undergo or have received an organ transplant.

Structural Elucidation and Characterization of the AGP Composition

Determination of Carbohydrate Content of the AGP Composition

Total carbohydrate content of the AGP compositions, of the present invention was determined by phenol-sulphuric acid method using D-galactose as a standard. The AGP composition, AGP-HM and AGP-LM were dissolved separately in a concentration of 0.16 mg/ml with distilled water. To 1 ml each of the three solutions taken separately was added 1 ml of 5 % phenol solution and 10 ml of sulphuric acid. These were vortexed to mix, allowed to cool till room temperature. Absorbance was measured at 480 nm. The carbohydrate content of the AGP compositions was found to be between 45-98 % respectively as compared with the galactose standard.

Determination of Protein Content of the AGP Composition

Total protein content of the AGP compositions, of the present invention was determined by Bradford method. Protein content of the AGP composition, AGP-HM and AGP-LM was between 2-20 %. The amino acid constituents of the AGP

composition were identified as asparagine, glutamine, hydroxyproline, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, lysine and phenylalanine, by amino acid analyzer using standards.

Identification of Glycosyl and Amino Acid Content of the AGP Composition by Hydrolysis and TLC 20 mg of neutral fraction obtained after size exclusion chromatography was taken and hydrolyzed with 2M trifluoro acetic acid (TFA, 200 µl) in a closed vial. The vial was heated at 100°C for 1 hr, concentrated and lyophilized. A Thin Layer Chromatography (TLC) of the mixture was run with standard sugar and amino acid samples. The results observed showed the presence of monosaccharides such as arabinose, galactose, glucose, rhamnose and mannose. Amino acids such as valine, phenylalanine, serine, GABA, isoleucine and histidine were also observed.

Identification of Glycosyl Content of the AGP Composition

Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-*O*-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the AGP-HM a and AGP-LM by acidic methanolysis.

Methyl glycosides were first prepared from dry sample of AGPs by methanolysis in 1 M HCl in methanol at 80°C (18-22 hours), followed by re-*N*-acetylation with pyridine and acetic anhydride in methanol (for detection of amino sugars). The samples were then per-*O*-trimethylsilylated by treatment with Tri-Sil (Pierce) at 80°C (0.5 hours). These procedures were carried out as previously described by York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., and Albersheim, P., *Methods Enzymol.*, 1985, 230, 1-15 and *Methods Enzymol.*, 1985, 118, 3-40.

GC/MS analysis of the TMS methyl glycosides was performed on an HP 5890 GC interfaced to a 5970 MSD, using an All Tech EC-1 fused silica capillary column (30m × 0.25 mm ID).

Table I summarizes the glycosyl components of AGP composition.

Table I

Glycosyl components of AGP composition

Components	*AGP Composition (Mole %)*
Arabinose (Ara)	34.00
Rhamnose (Rha)	3.40
Methylated uronic acid (MUA)	5.00
Mannose (Man)	2.20
Galactose (Gal)	48.90
Glucose (Glc)	Not Detected
Unidentified sugars	6.50

Determination of Glycosyl Linkage of AGP Composition

NaOH method: For glycosyl linkage analysis, a sample of AGP composition was permethylated, depolymerized, reduced, and acetylated; and the resultant partially methylated alditol acetates (PMAAs) analyzed by gas chromatography-mass spectrometry (GC-MS) as described earlier [York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., and Albersheim, P., *Methods Enzymol.*, 1985, 230, 1-15 ; York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., and Albersheim, P., *Methods Enzymol.*, 1985, 118, 3-40].

Initially, an aliquot of the sample was permethylated [by the method of Ciukanu and Kerek, *Carbohydr. Res.*, 1984, 131, 209-217] including treatment with sodium hydroxide and methyl iodide in dry DMSO. The permethylation was repeated twice in order to aid complete methylation of the polymer. Following sample workup, the permethylated material was hydrolyzed using 2 M trifluoroacetic acid (2 h in sealed tube at 121°C), reduced with $NaBD_4$, and acetylated using acetic anhydride/pyridine. The resulting PMAAs were analyzed on a Hewlett Packard 5890 GC interfaced to a 5970 MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m Supelco 2330 bonded phase fused silica capillary column.

Table-II summarizes the glycosyl linkages with percentages.

Table II

Glycosl Linkage of AGP Composition

Glycosyl Residue	*Percentage Present in the AGP Composition*
terminal rhamnopyranosyl residue (t-Rha *p*)	3.00
terminal arabinofuranosyl residue (t-Ara *f*)	22.00
terminal arabinopyranosyl residue (t-Ara *p*)	1.00
Mixture 3-rhamnopyranosyl residue (3-Rha *p*) 4-rhamnopyranosyl residue (4-Rha *p*)	1.00
terminal glucopyranosyl residue (t-Glc *p*)	3.00
terminal galactopyranosyl residue (t-Gal *p*)	5.00
5-linked arabinofuranosyl residue (5-Ara *f*)	5.00
2-linked mannopyranosyl residue (2-Man *p*)	Trace
2-linked glucopyranosyl residue (2-Glc *p*)	Trace
3-linked galactopyranosyl residue (3-Gal *p*)	16.00
4-linked galactopyranosyl residue (4-Gal *p*)	1.00
6-linked glucopyranosyl residue (6-Glc *p*)	2.00
6-linked galactopyranosyl residue (6-Gal *p*)	8.00
3,6-linked glucopyranosyl residue (3,6-Glc *p*)	2.00
3,6-linked galactopyranosyl residue (3,6-Gal *p*)	31.00

Determination of Molecular Weight of the AGP Composition

AGP composition, AGP-HM and AGP-LM were analyzed using Waters aqueous GPC instrument, Model Alliance 2690 which is equipped with integrated solvent and sample management unit and refractive index detector containing thermally shielded flow cell & optics with counter current heat exchanger for better baseline stability. The chromatography workstation includes data acquisition and control software and Millenium software for data processing. The GPC columns are from

Tosoh Corporation (TSK-GEL PW Type) consisting of hydrophilic polymer based semi rigid gel. The exclusion limit for polysaccharides (Dextran) is from 1,000 to 7,000,00 molecular weight. These columns were designed for analytical and preparative separation of synthesized water soluble polymers, oligomers and biological substances such as polysaccharides, nucleic acids, proteins, peptides, etc. The pullulan kit consists of polysaccharide samples of different molecular weight for column calibration.

A GPC method has been developed using varying analytical conditions such as different porosity columns, concentration & nature of mobile phase, flow rate, detector sensitivity etc. The optimum separation with polysaccharide sample is obtained by using 0.2 M sodium nitrate solution and the following were suitable analytical conditions for the characterization of polysaccharide based drug molecule.

The molecular weight of AGP composition was determined by comparison of the elution time using size exclusion chromatography. Based on the comparison the average molecular weight range of AGP composition was found to be between 10KD to 150 KD.

The molecular weights of AGP-LM and AGP-HM were also determined in a similar way. Based on the comparison the average molecular weight of the components were found to be 13 KD and 115KD, respectively.

Fourier Transform Infrared (FT-IR) Spectrum of the AGP Composition

IR spectrum of the AGP-HM of the present invention measured by Fourier Transform Infrared Spectrometer (8201 PC Shimadzu) using Nujol mull in KBr pellets shows broad absorption band at 3400 cm^{-1} indicating the presence of hydroxyl groups and at 2850-2960 cm^{-1} revealing the stretching of CH bonds.

h) ^1H Nuclear Magnetic Spectrum of the AGP Composition

^1H NMR spectrum of the AGP of the present invention was recorded in D_2O at 500 MHz in Bruker DRX 500 Nuclear Magnetic Resonance Spectrometer.

An extensive proton assignment of all the major linkage types detected by methylation analysis was made. In the ^1H NMR spectrum H-1 signals corresponding to the β-D-Gal-*p* residues (δ 4.22-4.47) and α-L-Ara-*f* residues (δ 5.17-5.33) were detected. There were numerous signals from δ 3.53-4.22, which corresponded to H-2 to H-6 of β-D-Gal *p* residues, H-2 to H-5 of the α-L-Ara *f* residues and H-2 to H-5 of small residues of glucose, rhamnose and mannose. To resolve and assign these signals

to particular residues 2D homo- and heteromuclear experiments were performed. Assignment of the different linkage types to each of the spin systems identified in the 2D spectra was supported both by the linkage data in Table II and by comparison with the NMR data reported in literature for other AGPs.

Anomeric protons : The anomeric protons observed at δ 5.33 and δ 5.17 were assigned to terminal and 5-linked α-L-Ara *f* residues, respectively. Signal at δ 5.17 was also assigned to terminal α-L-Rha *p* and δ 5.10 to 4-linked α-L-Rha *p*. Similarly, the signal at δ 4.61 was assigned to 3-linked α-L-Rha *p*. The signal at δ 4.56 was assigned to 6-linked β-D-Gal *p*. Signal at δ 4.61 was assigned to 3-linked β-D-Gal *p* and the signals at δ 4.56 and 4.57 were assigned to terminal β-D-Gal *p* and 3-, 6-linked β-D-Gal *p*.

H-2 protons : Signals at δ 4.31 and 4.22 were assigned to H-2 of terminal and 5-linked α-L-Ara *f* residue, respectively. Further, signal at δ 3.45 was assigned to terminal β-D-Gal *p* while the signal at δ 3.74 was assigned to 3-, 6-and 3,6- linked β-D-Gal *p* residues. Signals at δ 4.03, 3.74 and 3.57 were assigned to H-2 protons of 4-linked, 3-linked and terminal α-L-Rha *p*, respectively.

H-3 protons : Signal at δ 4.03 was assigned to terminal and 5-linked α-L-Ara *f* residues. Further the signal at δ 3.81 was assigned to terminal and 6-linked β-D-Gal *p*. Similarly signal at δ 4.31 was assigned to 3- and 3,6-linked β-D-Gal *p* moieties. Signals at δ 3.93, 3.63 and 3.57 were assigned to 4-linked, terminal and 3-linked α-L-Rha *p* respectively.

H-4 protons : Signal at δ 4.22 was assigned to terminal and 5-linked α-L-Ara *f*. Signal at δ 3.63 was assigned to 3- and 6-linked β-D-Gal *p* residues. Further, signals at δ 3.86 and 4.03 were assigned to terminal and 3, 6-linked β-D-Gal *p*, respectively. Similarly, signals at δ 3.63, 3.09 and 2.82 were assigned to 4-linked, 3-linked and terminal α-L-Rha *p* moiety.

H-5 protons : Signal at δ 3.81 was assigned to 6- and 3, 6-linked β-D-Gal *p*. Similarly signal at δ 4.03 was assigned to terminal and 3-linked β-D-Gal *p*. Signals at δ 3.91 and 3.93 were assigned to terminal and 5-linked α-L-Ara *f* respectively. Signals at δ 4.22, 4.03 and 3.09 were assigned to H-5 of terminal, 4-linked and 3-linked α-L-Rha *p*. Signals at δ 4.03, 3.45 and 4.03 could be assigned to H-5 of 3-linked β-D-Gal *p* and terminal β-D-Gal *p* residues, respectively.

H-6 protons : Signal at δ 3.93 was assigned to 6- and 3, 6-linked β-D-Gal *p*. Signals at δ 3.91 and 3.81 were assigned to terminal and 3-linked β-D-Gal *p*, respectively. The methyl protons observed at δ 1.03, 1.34 and 1.40 were assignable to 3-linked, 4-linked and terminal α-L-Rha *p* moiety.

The above assignments were further confirmed by carrying out HOMOCOSY experiments.

Amino acid residues : The methyl group for the amino acids valine, leucine and isoleucine were observed at δ 1.03 while the methyls assignable to threonine and alanine were located at δ 1.34 and 1.40, respectively.

Cα, Cβ (C-H, CH_2), Cγ (CH_2) and Cδ (CH_2) protons of arginine, asparagine, glutamine, isoleucine, leucine, lysine, methionine, proline and valine were observed between δ 1.3-2.8.

C-β (CH_2, CH), Cγ, Cδ protons of asparagine, histidine, phenylalanine and tyrosine were observed between δ 2.8-3.5.

Cα protons of alanine, arginine, glutamine, glycine, isoleucine, leucine, lysine, methionine, threonine and valine were observed between δ 3.5-3.9.

Cα protons of asparagine, histidine, phenylalanine, proline, serine, tyrosine and Cβ protons of serine and threonine were observed between δ 3.90-4.2.

Weak signals in the downfield region from δ 6.6–7.5 were assigned to N-H and aromatic protons of phenylalanine and tyrosine.

i) ^{13}C Nuclear Magnetic Spectrum of the AGP Composition

Anomeric Carbons : In the ^{13}C NMR spectrum the signals at δ 109.10 and 107.37 were assigned to the anomeric carbons of terminal and 5-linked α-L-Ara *f* moiety. Signals between δ 103 –102 were assigned to anomeric carbons of terminal, 3-linked, 6-linked and 3, 6-linked of β-D-Gal *p* residues. The same overlapping signals between δ 103-102 were assigned to anomeric carbons of 4-linked β-D-Glu *p*, 4-linked β-D-xyl *p*, terminal β-D-Glc *p* and 3-linked α-L-Rha *p*.

C-2 : The signals at δ 81.97 was assigned to 5-linked α-L-Ara *f* moiety. Signals for C-2 observed at δ 72.94 and 72.72 were assigned to terminal and 6-linked β-D-Gal *p* residues, respectively. Signal at δ 72.56 was assigned to 3- and 3,6-linked β-D-Gal *p* residue. Similarly, C-2 signals assignable to terminal and 6-linked β-D-Glc *p* were observed at δ 74.81, 74.60 and 73.33, respectively. The C-2 signals in α-L-Rha *p*

residues assignable to terminal, 3-linked and 4-linked were observed at δ 80.02, 76.55 and 70.68, respectively. The signal at δ 81.24 was assigned to 2-linked β-D-Man *p* residue.

C-3 : Signal at δ□76.55 was assigned to terminal and 5-linked α-L-Ara *f*.□ Signal at δ74.81 was assigned to terminal and 6-linked β-D-Gal *p*. Further, the signal at δ 81.97 was assigned to 3- and 3,6-linked β-D-Gal *p* residue. The signals at δ 76.55 and 74.81 were assigned to terminal, 4-linked and 6-linked β-D-Glc *p* moieties. Similarly, the signals at δ 80.02, 76.55 and 70.6 were assigned to terminal, 3-linked and 4-linked α-L-Rha *p* moiety, respectively.

C-4 : Signal at δ 83.85 was assigned to terminal and 5-linked α-L-Ara *f* moiety. Signal at δ 70.15 was assigned to terminal β-D-Gal *p* residue. Signal at δ 70.68 was assigned to 3- and 6-linked β-D-Gal *p* residue. While the signal at δ 73.33 was assigned to 3, 6-linked β-D-Gal *p* residue. Signals at δ 80.02, 70.68 and 70.15 were assigned to C-4 of 6-linked and terminal β-D-Glc *p* moiety, respectively. Signals at δ 81-83 were assigned to C-4 of 3-linked, 4-linked and terminal α-L-Rha *p* moiety.

C-5 : Signal due to C-5 of 5-linked α-L-Ara *f* was observed at δ 69.17 while that of terminal α-L-Ara *f* was observed at δ 61.25. C-5 signals due to 3-linked, 6-linked and terminal β-D-Gal *p* residue were observed between δ 74-77, whereas, the C-5 signals due to terminal was observed at δ 75-76 in the case of β-D-Glc *p* moiety.

C-6 : The C-6 signals due to 6- and 3, 6-linked β-D-Gal *p* residues were observed at δ 69.17 whereas 3-linked- and terminal signals were observed at δ 60.93 and 61.25, respectively. Signal due to C-6 in β-D-Glc *p* residues for terminal was observed between δ 60-62 and the signal at δ 70.1 was observed for 6-linked.

Methyls : The signals observed at δ 16.7, 19.9 and 22.2 were assigned to methyl groups of α-L-Rha *p*.

The above assignments were further confirmed by carrying out HETCOR experiments.

Amino acids : Signal at δ 16.70 was assigned to C γ and Cδ methyls of valine, Cβ methyl of alanine and Cδ □methyl of methionine. Signal at δ 19.97 was assigned to Cγ methyl of threonine. Signal at δ 22.22 was assigned to Cγ (CH_2), Cγ (C-H) of arginine, leucine, isoleucine, lysine and to Cδ and Cε methyls of leucine.

The signal at δ 31.01 was assigned to Cβ (CH_2) of arginine, methionine, lysine, proline and Cγ (CH_2) of glutamine.

Signal at δ 38.05 was assigned to Cβ (CH_2) of asparagine, leucine, phenylalanine, tyrosine, Cα of glycine and Cδ (CH_2) of arginine and lysine. Signal at δ 48.45 was assigned to Cα proton of alanine, asparagine, leucine and Cδ of proline.

Signal at δ 59.81 was assigned to Cα protons of arginine, cystine, glutamine, histidine, isoleucine, lysine, methionine, phenylalanine, serine and tyrosine. Signal at δ 60.9 was assigned to Cα proton of valine and threonine while the signal at 61.25 was assigned to Cα proton of proline and Cβ proton of serine.

The signal at δ 68.38 was assigned to Cβ (CH) of threonine.

Signals between δ 108-140 were assigned to the olefinic protons of histidine and aromatic protons of phenylalanine.

Signal at δ 155.64 was assigned to Cε of arginine and C-4 of tyrosine whereas carbonyl signal at δ 166.19 was assigned to glutamine.

In the downfield region, the signals observed between δ 171 – 175 were assigned to the carbonyl groups of the amino acids alanine, arginine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine and tyrosine.

In conclusion the structure of AGP-HM consists of 6-linked- galactopyranose and 3,6-linked galactopyranose as the backbone and arabinofuranosyl, arabinopyranosyl, rhamnopyranosyl, glucopyranosyl and galactopyranosyl residues in terminal positions. The AGP further contains 3-linked, 4-linked □rhamnopyranosyl, 2-linked-mannopyranosyl, 2-linked-glucopyranosyl, 3-linked-, 4-linked galactopyranosyl, 6-linked-, 3,6-linked-glucopyranosyl and 4-linked-xylopyranosyl residues along with methyl uronic acid. The amino acid constituents of the AGP composition were identified as asparagine, glutamine, hydroxyproline, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, lysine and phenylalanine. However, sites of attachment of amino acids have not been determined. AGP-LM also possesses similar structure but differs in the molecular weight. Based on the aforesaid data the following structure has been proposed for AGP composition (Fig. 2). Both AGP-HM and AGP-LM possess similar biological activity, hence, AGP composition has been employed for all biological studies.

The Immunological and Pharmacological Properties of the AGP Composition

The cytokine assay, such as IL-2, IFN-γ, IL-10 and other *in vivo* activities such as delayed type hypersensitivity (DTH) in guinea pigs are described below. A comparison of biological activities of aqueous extract as obtained by the method disclosed in US Application Publication No. 2003/0194456 A1 and AGP composition of the present invention is given in Table-III.

Table III

Comparative Biological Efficacy of Aqueous Extract (1) obtained as per the Method disclosed in US Application Publication No. 2003/0194456 A1 ***and the AGP Composition of the Present Invention (2)***

Sr. No.	*Parameters*	*Aqueous Extract (1)*	*AGP Composition (2)*
1	IL-10 induction (EC_{50} in µg/ml)	2.54	1.075
2	IL-2 inhibition (% inhibition at 20ng/ml)	29.32±5.26	51.10±4.33
3	IFNγ inhibition (% inhibition at 20ng/ml)	33.27±6.52	54.50±4.00
4	TNFα inhibition (% inhibition at 2 µg/ml)	44.34±6.64	60.68±9.03
5	GMCSF inhibition (% inhibition at 200ng/ml)	35.57±9.45	43.28±6.21
6	TPA induced skin hyperplasia (ED_{50}mg/kg)	36.3	2.31
7	DTH Model (ED_{50} mg/kg)	4.34	0.58
8	MEST (ED_{50} mg/kg)	14.0	6.43

Toxicological Studies on the AGP Composition

Acute toxicity (LD_{50}) of AGP composition was evaluated in mice and rat by oral and i.v. routes. Group of ten (10) animals from each species per route per dose were medicated and results were calculated on day 15.

The following values were observed for the AGP composition

LD_0 of mice p.o.	:	>5000mg/kg b.wt.
LD_0 of mice i.v.	:	>1000mg/kg b.wt.
LD_0 of rat p.o.	:	>5000mg/kg b.wt.
LD_{50} of rat i.v.	:	> 250mg/kg b.wt.

Biological Assay of the AGP Composition

Description of the Methods of Evaluation for Human IL-10 Production

In order to evaluate the efficacy of the AGP composition for its therapeutic potential in psoriasis, its role in *in vitro* IL-10 induction was evaluated by IL-10 production assay with ConA induced PBMCs [Raychudhuri S. P., Farber E. M., Raychudhuri S. K., *Int. J. Immunopharmacol.*, 1999, 21,609].

Briefly, Human PBMCs (Peripheral blood homonuclear cells) were separated out and stimulated with 10 µg/ml ConA along with various concentrations of AGP composition and incubated for 48 hours at 37°C in CO_2 incubator with 5% CO_2. The supernatants were harvested and frozen at –70°C until quantitation by ELISA. Human IL-10 ELISA kits used were from R and D System for detection of IL-10 in culture supernatant. Percent induction was calculated with reference to control. The results are summarized in Table-IV.

The AGP composition isolated from *Argemone mexicana* exhibited increase in production of IL-10 in ConA activated human PBMCs in the range of 0.0002 µg/ml to 200 µg/ml (Fig. 3). IL-10 was found to be regulatory cytokine in psoriasis treatment and is well known for inducing anti-psoriatic therapy.

IL-10 induction is useful in the treatment and/or prophylaxis of psoriasis, dermatitis, scleroderma, inflammatory disorders and other autoimmune diseases like psoriatic arthritis, plaque psoriasis, guttate psoriasis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, irritable bowel disease, ankylosing spondilitis, systemic lupus erythrometosus, Sjogren's syndrome, allergies like asthma, chronic obstructive pulmonary disease and related conditions such as eczema and scaly itchy patches. IL-

10 induction is also useful in other chronic, recurrent and other skin ailments where cutaneous lymphocyte antigen or cutaneous leukocyte antigen is involved.

Table IV

Effect of the AGP Composition on IL-10 Induction by ConA induced Human PBMCs

Concentration of Extract (µg/ml)	*Average % Induction*
200	371.40
20	303.80
2	237.30
0.2	137.20
0.02	114.30
0.002	106.00
0.0002	102.60

*The values are depicted in percent increase from basal with reference to control.

Description of the Methods of Evaluation for Human IL-2 and IFN-γ Production

In order to evaluate the efficacy of AGP composition for its therapeutic potential in psoriasis, its role in *in vitro* IL-2 and IFN-γ modulation was evaluated by IL-2 and IFN-γ production inhibition assay with phytohemagglutin (PHA) induced PBMCs [Brynskov J, Tvede N., *Gut.*, 1990, 31(7), 795].

The aim of this study was to evaluate the effect of AGPs on PHA induced IL-2 and IFN-γ production from human lymphocyte. Briefly, peripheral blood mononuclear cells (PBMC) were obtained from healthy individuals. One million PBMC per ml were stimulated with PHA (5µg/ml) along with various concentrations of AGP composition isolated from *Argemone mexicana* for 48 hours at 37 °C in CO_2 incubator with 5% CO_2. The supernatants were harvested and frozen at –70°C. Human IL-2 and Human IFN-γ ELISA kits used were from R and D System for detection of IL-2 and IFN-γ in culture supernatant. Percent inhibition was calculated with reference to control.

AGP composition isolated from leaves and/or stems of *Argemone mexicana* was found inhibitory to mitogen induced IL-2 production between 0.002µg/ml to 20µg/ml (Fig. 4).

This inhibitory activity to mitogen IL-2 production is known to be immunosuppressive and well established to be useful in treatment and/or prophylaxis of psoriasis. The results are summarized in Table-V.

Table V

Effect of AGP Composition on IL-2 Production by PHA induced Human PBMCs

Concentration of AGP Composition(µg/ml)	*Average % Inhibition*
20.00	54.53
2.00	54.49
0.20	53.97
0.02	51.10
0.002	30.56
0.0002	10.87
0.00002	3.75

*The values are depicted in percent inhibition with reference to control.

The AGP composition isolated from *Argemone mexicana* was found inhibitory to mitogen induced IFNγ production in the range of 0.0002µg/ml to 2µg/ml (Fig. 5). This inhibitory activity to mitogen induced IFN-γ production is known to be immunosuppressive and well established to be useful in treatment and/or prophylaxis of psoriasis. The results are summarized in Table VI.

Table VI

Effect of AGP Compositions on IFN-γ Production by PHA induced Human PBMCs

Concentration of AGP Composition(µg/ml)	*Average % Inhibition*
20.00	55.19
2.00	57.07
0.20	53.69
0.02	54.50

0.002	38.62
0.0002	15.34
0.00002	4.04

*The values are depicted in percent inhibition with reference to control.

Description of the Methods of Evaluating for Human GMCSF and TNF-alpha Production

In order to evaluate the efficacy of the AGP compositions of this invention for its therapeutic potential in psoriasis, its role in *in vitro* granulocyte macrophage colony stimulating factor (GMCSF) and TNF-α modulation was evaluated by GMCSF and TNFγ production inhibition assay with PBMCs stimulated by ConA and LPS.

Briefly, Human PBMCs were separated from blood of healthy volunteers, stimulated with 10 µg/ml ConA along with various concentrations of AGP composition and incubated for 48 hours at 37°C in CO_2 incubator with 5% CO_2. 5µg/ml of LPS was added and incubated for 24 hours under the same conditions. The supernatants were harvested and frozen until quantitation of cytokines using ELISA. Percent inhibition was calculated with reference to control.

The AGP composition isolated from *Argemone mexicana* was found inhibitory to mitogen induced GMCSF production in range of 0.02µg/ml to 2µg/ml (Fig. 6). This inhibitory activity to mitogen induced GMCSF production is known to be immunosuppressive and well established to be useful in treatment and/or prophylaxis of psoriasis. The results are summarized in Table-VIII.

Table VII

Effect of AGP Composition on GMCSF Production by ConA and LPS induced Human PBMCs

Concentration of AGP Composition(µg/ml)	*Average % Inhibition*
2.00	32.31
0.20	43.28
0.02	35.41

*The values are depicted in percent inhibition with reference to control.

AGP composition isolated from *Argemone mexicana* was found inhibitory to mitogen induced TNF-α production in range of 0.002μg/ml to 2μg/ml (Fig. 7). This inhibitory activity to mitogen TNF-α production is known to be immunosuppressive and well established to be useful in treatment and/or prophylaxis of psoriasis. The results are summarized in Table VIII.

Table VIII

Effect of AGP Compositions on TNF-α production by ConA and LPS induced Human PBMCs

Concentration of AGP composition(μg/ml)	*Average % Inhibition*
2.00	60.68
0.20	46.18
0.02	22.02
0.002	10.30

*The values are depicted in percent inhibition with reference to control.

Effect of the AGP Composition on NGF Induced Human Keratinocytes Proliferation

Keratinocytes were purchased from GIBCO (USA) and were maintained in Keratinocyte-serum free medium (GIBCO, # 10744-019) supplemented with growth factors. Approximately, 150 μl of KGM (keratinocyte growth medium) containing 2000 cells were added in each well of 96 well flat bottom plate. Next day medium was changed with 1:2 volume of KGM: KBM (keratinocyte basal medium). Thirty microliters of diluted AGP composition was added in each well in triplicate except cell control wells. NGF (Nerve growth factor) 100 ng/ml was added in each well. Medium was removed after 8 days of incubation. Cells were rinsed with PBS. LDH (Lactate dehydrogenase) development lysis solution was added and incubated for 10 min at 37°C. OD was measured in ELISA reader at wavelength of 492 nm. Percent proliferation/ inhibition was calculated. The results are summarized in Table-IX

Table IX

Effect of AGP Composition on NGF Induced Human Keratinocytes Proliferation

AGP Composition (µg/ml)	*Average % Inhibition*
40.00	79.15
8.00	74.53
1.60	71.49
0.32	66.78
0.064	55.75
0.0128	26.78
0.0025	15.36
0.0005	6.18
0.0001	3.03

*The values are depicted in percent inhibition with reference to control.

The AGP composition isolated from *Argemone mexicana* was found inhibitory to NGF induced human keratinocytes proliferation in range of 0.0001µg/ml to 40µg/ml (Figs. 8 & 9). This inhibitory activity to NGF induced human keratinocytes proliferation is known to be immunosuppressive and well established to be useful in treatment and/or prophylaxis of psoriasis.

Description of the Methods of Evaluation of *in vivo* Immunosuppression using Delayed Type Hypersensitivity in Guinea pigs

This standard procedure was used for evaluation of the *in vivo* efficacy of AGP composition for its ability to inhibit Purified protein derivative (PPD) induced delayed type hypersensitivity in guinea pigs. Briefly, guinea pigs were sensitized with 100 µg of PPD intradermally with Freund's complete adjuvant. Two subsequent boosters of 100 µg of PPD with Freund's incomplete adjuvant were given at a week interval. AGP composition was administered orally once a day for 28 days. The animals were challenged with 100 µg of PPD intradermally on 28th day and the difference in the control and PPD injected skin thickness was measured after 24 hours post challenge with a Varnier's caliper. The differences of skin thickness were calculated by

subtracting saline injected skin thickness in same guinea pigs. Percent inhibition was calculated with reference to saline sensitized animals.

The AGP composition isolated from *Argemone mexicana* was found to be immunosuppressive to PPD sensitized and PPD challenged guinea pigs in the range of 20-65.89 % inhibition at the dose of 0.10 – 100mg/kg p.o. Effective dose causing 50% inhibition (ED_{50}) of skin thickness in PPD challenged guineapigs was found to be 0.508mg/kg p.o (Fig. 10). The potent immunosuppressive property is well established and is beneficial for anti-psoriasis treatment.

Immunosuppression in DTH model is a useful model for several diseases where immunosuppression is required, such as psoriasis, dermatitis, scleroderma, inflammatory disorders and other autoimmune diseases like psoriatic arthritis, plaque psoriasis and guttate psoriasis. The results are summarized in Table-X.

Table X

Effect of the AGP composition on skin thickness in guine pigs challenged with PPD

Concentration of AGP Composition mg/kg	*Percent Response*
0.01	0.000
0.10	20.000
1.00	62.680
10.00	67.930
30.00	61.510
100.00	65.880

Description of the Methods of Evaluation of TPA

Balb/C mice were randomized and acclimatized in cages 5 days before the experiment. Hair were removed by using Anne French topical application and cleaned properly. Groups were made consisting of six mice in each. Acetone control group and TPA (12-O-tetradecanoylphorbol-13-acetate) control groups were taken along with the test drug group. AGP composition was dissolved in the recommended vehicle (acetone) and animals were dosed orally for 4 days once daily. Twenty microliter of 100nM TPA

was applied on the second day onto the cleaned skin surface and allowed to be absorbed. Animals were sacrificed 72 hours after TPA application. The skin pieces were fixed in 10% formalin for 3 days. Histopathological slides were made by following standard methods. Epidermal thickness was measured in at least 10 different areas by using a oculometer and results were expressed in percent inhibition (Fig. 11). The results are summarized in Table-XI.

Table XI

Effect of the AGP Composition on TPA Model

Groups	*Dose (mg/kg,p.o.)*	*Increase in Epidermal Thickness (μm)*	*% Inhibition*
Acetone control group	-	23.08	-
TPA control group	-	52.88	0.00
AGP Composition	30.00	25.38	92.30
	10.00	29.67	77.89
	3.00	35.63	57.90
	1.00	50.08	9.37
	0.30	52.63	0.84
	0.10	52.67	0.70

*The values are depicted in percent inhibition with reference to TPA control group.

Description of the Methods of Evaluation of *in vivo* Immunosuppression using Mouse Ear Swelling Test (MEST)

This standard procedure was used for evaluation of the *in vivo* efficacy of AGP compositions for their ability to inhibit DNFB induced delayed type hypersensitivity in mice [Cornacoff J. B, House R. V, Dean J. H., *Fundam. Appl. Toxicol.*, 1988, 10(1), 40 ; Fundam. Appl. Toxicol., 1992, 19(1), 157].

Briefly, C57BL6 mice were used for the test. Mice were sensitized with 0.2% DNFB (in 1:4 of Olive oil and Acetone) on back of the mice. Three boosters DNFB application were done every third day. The mice were challenged with 0.2% DNFB (in 1:4 of Olive oil and Acetone) on ear pinna. Ear thickness in the center of the ear was measured after 24 hours with the help of Varnier caliper. The analysis was performed,

by calculating percent inhibition with respect to a negative control were given orally at different doses. The results are summarized in Table-XII.

Table XII

Effect of AGP Composition on DNFB induced Mice Ear Swelling Test in Female C57/BL6 Mice

Sr. No.	Treatment	Percent Inhibition
1	Milli Q water 10 ml/kg, p.o. Negative control for AGP composition	0.00
2	2% Tween 80, 10 ml/kg, p.o. Negative control for Cyclosporin	0.00
3	AGP Composition - 0.1 mg/kg, p.o.	0.00
4	AGP Composition - 1 mg/kg, p.o.	18.00
5	AGP Composition - 3 mg/kg, p.o.	33.05
6	AGP Composition - 10 mg/kg, p.o.	43.62
7	AGP Composition - 30 mg/kg, p.o.	58.52
8	AGP Composition – 100 mg/kg, p.o.	69.51
9	Cyclosporine 25 mg/kg, p.o.	65.16

Data represented as percent inhibition with each from 7-12 animals. The AGP composition was compared with Milli Q water treated group and cyclosporine was compared with animals treated with 2% Tween 80

The AGP composition from the leaves and/or stem of Argemone *mexicana* were found to be immunosuppressive to DNFB sensitized C57BL6 mice. The ED_{50} for AGP composition was determined to be 6.43 mg/kg (Fig. 12). The potent immunosuppressive property is well established and beneficial for treatment or prophylaxis of psoriasis.

Immunosuppression in the MEST model is useful in evaluating effect for several diseases where immunosuppression is required, such as psoriasis, dermatitis, scleroderma, inflammatory disorders and other autoimmune diseases like psoriatic arthritis, plaque psoriasis, guttate psoriasis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, ankylosing spondilitis, systemic lupus erythrometosus, Sjogren's

syndrome, allergies like asthma, chronic obstructive pulmonary disease and related conditions as eczema, scaly itchy patches.

The invention is further illustrated by the following non-limiting examples.

Example 1

Isolation of AGP Composition, AGP-HM and AGP-LM

10 Kg fresh leaves of *Argemone mexicana* were ground and extracted with demineralised water (15 litres). The slurry was centrifuged and the aqueous extract was concentrated below 40°C under vaccum. The concentrated material was lyophilized to give 0.54 Kg of dry solid.

500 g of the dry aqueous extract was dissolved in 6 litres of water, centrifuged and decanted. The supernatant was loaded on a cation exchange column (5 litres) at the rate of 10 ml/min. The eluate (7.5 litres) was collected, concentrated (2.5 litres) and loaded on an anion exchange column (5 litres). The eluate (3 litres) was concentrated to approximately 1.5 litres. The experiment was repeated with another 0.5 kg of the material (aqueous extract).

The combined eluates (1.5 litres) each were mixed and concentrated to 2.5 litres and loaded on a cation exchange column. The eluate (3 litres) was concentrated up to 1.2 litres and loaded on an anion exchange column. The eluate (1.5 litres) was lyophilized to give 42.39 g of a powder.

42 g of the above powder was dissolved in 600 ml of water and partitioned between n-butanol (3 X 400 ml) and water. The n- butanol layer was discarded. 3 litres of methanol was poured in to the aqueous layer (600ml) when a solid precipitated out. The solution was centrifuged and the supernatant decanted. The supernatant was concentrated to a volume of ca. 300ml. Methanol (1 litre) was poured again to precipitate the methanol insolubles. The solution was centrifuged and the supernatant decanted. The precipitated solid from both experiments were lyophilized (8.75 g). The dry materials were dissolved in water, and subjected to XAD-2 column, twice. The water eluate was lyophilized to give 6.10 g of AGP composition. This was further subjected to sephacryl chromatography to yield 5 mg of AGP-HM and 10.3 mg of AGP-LM.

Example 2

Isolation of AGP composition, AGP-HM and AGP-LM

22 Kg of fresh leaves of *Argemone mexicana* were ground and extracted with demineralised water (36 litres). The slurry was centrifuged and the aqueous extract was concentrated below 40°C under vaccum. The concentrated material was lyophilized to give 1.25 Kg of dry solid.

1.2 kg of the dry aqueous extract was dissolved in 12 litres of water, centrifuged and decanted. The supernatant was loaded on a cation exchange column (5 litres) at the rate of 10 ml/min. The eluate (15.5 litres) was collected, concentrated (5.5 litres) and loaded on an anion exchange column (5 litres). The eluate (6.25 litres) was concentrated to approximately 3.2 litres.

The eluate (3.2 litres) was concentrated to 2.6 litres and loaded on a cation exchange column. The eluate (4 litres) was concentrated up to 1.5 litres and loaded on an anion exchange column. The eluate (1.6 litres) was lyophilized to give 54 g of a powder.

40 g of the above powder was dissolved in 700 ml of water and partitioned between n-butanol (3 X 500 ml) and water. The n-butanol layer was discarded. 4 litres of methanol was poured in to the aqueous layer (675ml) when a solid precipitated out. The solution was centrifuged and the supernatant decanted. The supernatant was concentrated to 400 ml. Methanol (3 litre) was poured again to precipitate the methanol insolubles. The solution was centrifuged and the supernatant decanted. The precipitated solids were lyophilized (7.3 g). The dry material thus obtained was dissolved in water, and subjected to XAD-2 column, twice. The water eluate was lyophilized to give 5.5 g of AGP composition. This was further subjected to sephacryl chromatography to yield 4.5 mg of AGP-HM 12.4 mg of AGP-LM.

Example 3

Table XIII

Unit Formulae for a Capsule Formulation Comprising the AGP of the Present Invention and Pharmaceutically Acceptable Carriers

Sr. No.	Ingredient	Strength (mg/capsule)	
01	AGP	50	200
02	Microcrystalline Cellulose USNF (Avicel PH 102)	39	39
03	Croscarmellose Sodium USNF (Ac-Di-SOL)	10	10
04	Colloidal Silicon Dioxide USNF (Aerosil 200)	0.5	0.5
05	Magnesium Stearate USNF	0.5	0.5
	Total	100	250
06	Hard gelatin capsule	1 (size '2' with orange body and orange cap)	1 (size '00' with orange body and red cap)

The above-mentioned ingredients given in Table-XIII can be blended until uniform and then filled in hard gelatin capsules of the size "00". The processing area is ideally maintained at 40±5% RH at 18-22E C.

A typical process for preparation of the composition of the invention is illustrated below:

The active ingredient, Microcrystalline Cellulose, Croscarmellose Sodium, Magnesium Stearate and colloidal silicon dioxide are blended until uniform. They are filled in hard gelatin capsules of size "00". The processing area is ideally maintained at 40±5% RH at 18EC to 22EC.

When a topical application is administered, the amount of the of *Argemone mexicana* plant ranges from 0.1% to 10% by weight of the extract.

A Unit Formuale for an ointment for topical application comprising the AGP and carriers is summarized in Table-XIV.

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Table-XIV

Unit Formulae for an Ointment for Topical Application Comprising the AGP of the Present Invention and Pharmaceutically Acceptable Carriers

Sr. No.	*Ingredient*	*Amount*
01	AGP	1.0 gm
02	Water	100 ml
03	Hydroxypropyl Methyl Cellulose	4.0 gm

A typical process for preparation of the ointment for topical application comprises slow blending of the all the ingredients to a smooth gel by conventional methods and filling into tubes.

CLAIMS

1. A purified Arabinogalactan-Protein (AGP) composition, having an average molecular weight range between 10 KD to 150 KD, isolated from the leaves and/or stems of *Argemone mexicana* plant obtainable by a process comprising the steps of:

i) extracting 1 part by weight of the leaves and/or stems of *Argemone mexicana* plant with 1 to 10 parts by weight of water, a C_{1-3} alcohol or mixtures thereof to obtain an aqueous extract; and partially or completely concentrating or lyophilizing the extract;

ii) subjecting the aqueous extract, partially concentrated aqueous extract or an aqueous solution of the completely concentrated or lyophilized extract as obtained in step i) successively to ion exchange chromatography to obtain a neutral aqueous extract;

iii) fractionating the neutral aqueous extract obtained in step ii) with n-butanol and separating the aqueous and the n-butanol phases;

iv) mixing and agitating the aqueous phases obtained in step iii) with methanol or ethanol, and isolating the precipitated solids to obtain an insoluble fraction; and

v) subjecting the insoluble fraction obtained in step iv) to successive gel chromatography and size exclusion chromatography to obtain purified Arabinogalactan-Protein (AGP) composition.

2. The composition according to claim 1, wherein the C_{1-3} alcohol is selected from methanol, ethanol, 1-propanol or 2-propanol.

3. The composition according to claim 1, wherein the ion exchange chromatography is cation exchange chromatography followed by anion exchange chromatography.

4 The composition according to claim 1, wherein the ion exchange chromatography is anion exchange chromatography followed by cation exchange chromatography.

5.. The composition according to claim 3 or 4, wherein the cation exchange chromatography is carried out over sulphonated polystyrene strong-acid cation exchangers or carboxylic acid-type weak acid cation exchangers.

6. The composition according to claim 3 or 4, wherein the anion exchange chromatography is carried out over aliphatic amine-type weak base anion exchangers or strong base anion exchangers.

7. The composition according to claim 1, substantially free of other alkaloids, flavanoids, amino acids, organic acids, fatty acids, and other compounds present in the leaves and/or stems of the *Argemone mexicana* plant.

8 The composition according to claim 1, comprising 6-linked galactopyranose and 3,6-linked galactopyranose as the backbone and arabinofuranosyl, arabinopyranosyl, rhamnopyranosyl, glucopyranosyl and galactopyranosyl residues in terminal positions, and 3-linked□□-4-linked□ rhamnopyranosyl, 2-linked-mannopyranosyl, 2-linked-glucopyranosyl, 3-linked, 4-linked galactopyranosyl, 6-linked, 3,6-linked-glucopyranosyl, 4-linked-xylopyranosyl residues along with methyl uronic acid.

9. The composition according to claim 1, which is constituted of 45-98 % by weight of carbohydrates.

10. The purified Arabinogalactan-Protein (AGP) composition according to claim 1, which is constituted of 2-20 % by weight of proteins.

11. A method for isolating Arabinogalactan-Protein (AGP) composition, comprising the steps of:

i) extracting 1 part by weight of the leaves and/or stems of *Argemone mexicana* plant with 1 to 10 parts by weight of water, a C_{1-3} alcohol or mixtures thereof to obtain an aqueous extract and partially or completely concentrating or lyophilizing the extract;

ii) subjecting the aqueous extract, partially concentrated aqueous extract or an aqueous solution of the completely concentrated/lyophilized extract as

obtained in step i) successively to ion exchange chromatography to obtain a neutral aqueous extract;

iii) fractionating the neutral aqueous extract obtained in step ii) with n-butanol and separating the aqueous and the n-butanol phases;

iv) mixing and agitating the aqueous phases obtained in step iii) with methanol or ethanol, and isolating the precipitated solids to obtain an insoluble fraction; and

v) subjecting the insoluble fraction obtained in step iv) to successive gel chromatography and size exclusion chromatography to obtain purified Arabinogalactan-Protein (AGP) composition.

12. The method according to claim 11 wherein the C_{1-3} alcohol is selected from methanol, ethanol, 1-propanol or 2-propanol.

13. The method according to claim 11, wherein the ion exchange chromatography is cation exchange chromatography followed by anion exchange chromatography.

14. The method according to claim 11, wherein the ion exchange chromatography is anion exchange chromatography followed by cation exchange chromatography.

15. The method according to claim 13 or 14 , wherein the cation exchange chromatography is carried out over sulphonated polystyrene strong-acid cation exchangers or carboxylic acid-type weak acid cation exchangers.

16. The method according to claim 13 or 14 wherein the anion exchange chromatography is carried out over aliphatic amine-type weak base anion exchangers or strong base anion exchangers.

17. The method according to claim 11, wherein the gel chromatography is carried out over an Amberlite polymeric adsorbent.

18. The method according to claim 11, wherein the size exclusion chromatography is carried out over sephacryl.

19. The method according to claim 11, wherein the gel chromatography is carried out over an Amberlite polymeric adsorbents selected from XAD-2, XAD-4 and XAD-7.

20. The method according to claim 11, wherein the size exclusion chromatography is carried out over sephacryl selected from sephacryl S-100, sephacryl S-200 HR, and sephacryl S-300 HR.

21. A pharmaceutical composition comprising a therapeutically effective amount of the purified Arabinogalactan-Protein (AGP) as claimed in any one of claims 1 to 10 and a pharmaceutically acceptable excipient.

22. The composition according to claim 21, further comprising at least one of non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, or flavouring agents.

23. The pharmaceutical composition according to claim 21, wherein the pharmaceutically acceptable excipient is selected from sugars, starches, cellulose and its derivatives calcium phosphates. sodium sulphate; calcium sulphate; polyvinylpyrrolidone; polyvinyl alcohol; stearic acid; vegetable oils; non-ionic, cationic and anionic surfactants; ethylene glycol polymers; β-cyclodextrin; fatty alcohols; or hydrolyzed cereal solids.

24. A purified Arabinogalactan-Protein (AGP) having an average molecular weight range between 10 KD to 150 KD, isolated from the leaves and/or stems of Argemone mexicana plant.

25. A pharmaceutical composition compriaing a therapeutically effective amount of the purified Arabinogalactan-Protein (AGP) of claim 59 and a pharmaceutically acceptable excipient.

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26. A purified Arabinogalactan-Protein (AGP) having an average molecular weight range between 10 KD to 150 KD, isolated from the leaves and/or stems of *Argemone mexicana* plant

27. Use of a purified Arabinogalactan-Protein (AGP) as claimed in any one of claims 1 to 10, 21 or 26 in the preparation of a medicament for the treatment of psoriasis, inflammatory disorder, autoimmune disease , allergy, eczema or scaly itchy patches, or skin ailment in mammals.

28 Use of a composition as claimed in any one of claims 21 to 23 or 25 in the preparation of a medicament for the treatment of psoriasis, inflammatory disorder, autoimmune disease, allergy, eczema or scaly itchy patches, or skin ailment in mammals.

29. Use according to claims 24 or 25 wherein the disorder is selected from the group consisting of dermatitis; scleroderma; eczema; psoriatic arthritis, rheumatoid arthritis, Crohn's disease, multiple sclerosis, irritable bowel disease, ankylosing spondilitis, systemic lupus erythrometosus, Sjogren's syndrome, and scaly itchy patches.

30. Use according to claims 24 or 25 wherein said allergy is asthma or chronic obstructive pulmonary disease.

31. Use according to claims 24 or 25 wherein said psoriasis is selected from the group consisting of plaque psoriasis, guttate psoriasis, pustular psoriasis and psoriasis of the nails.

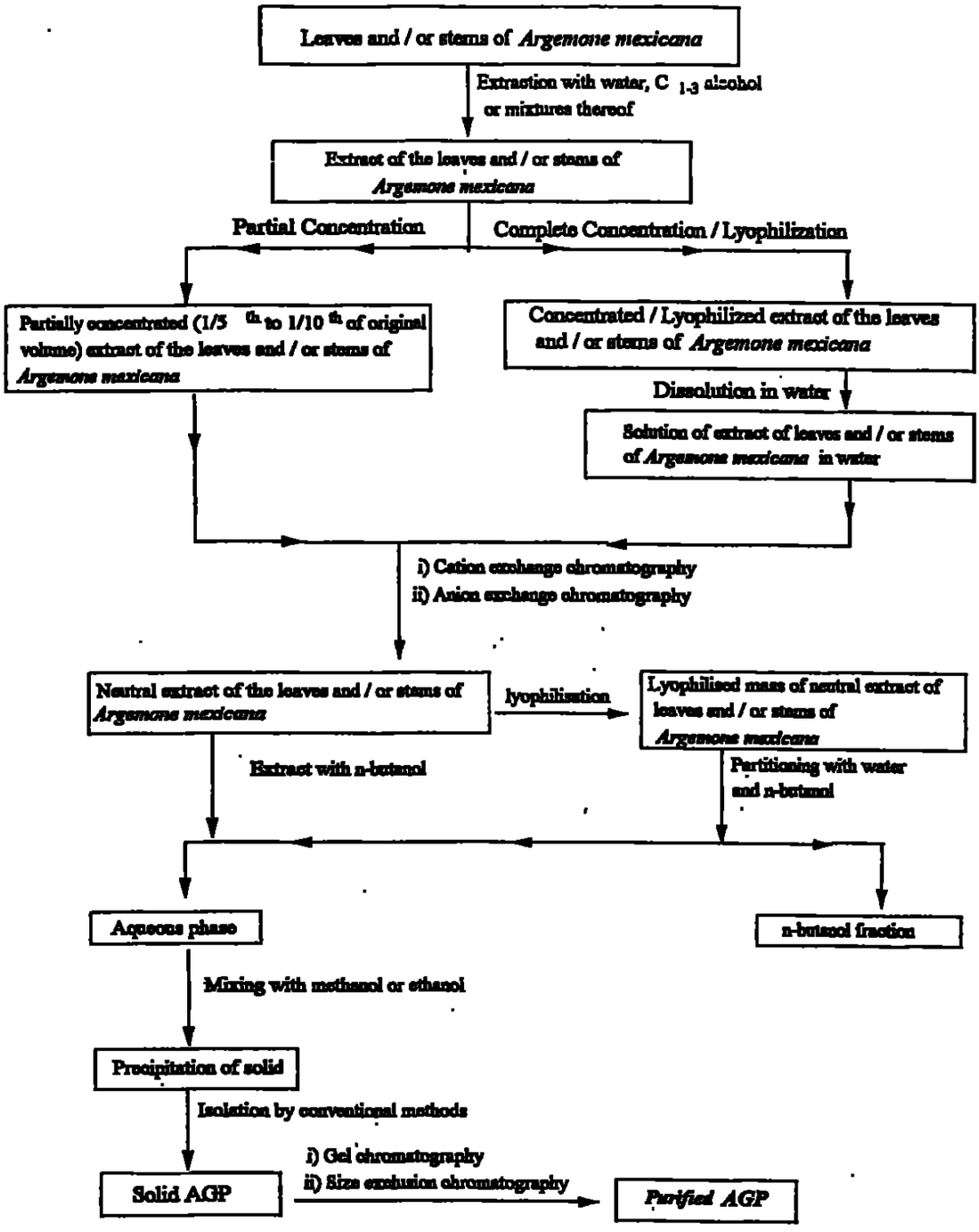

Fig. 1 : The Selective Method for Isolation of Purified Arabinogalactan-Protein (AGP) Composition as per the Present Invention

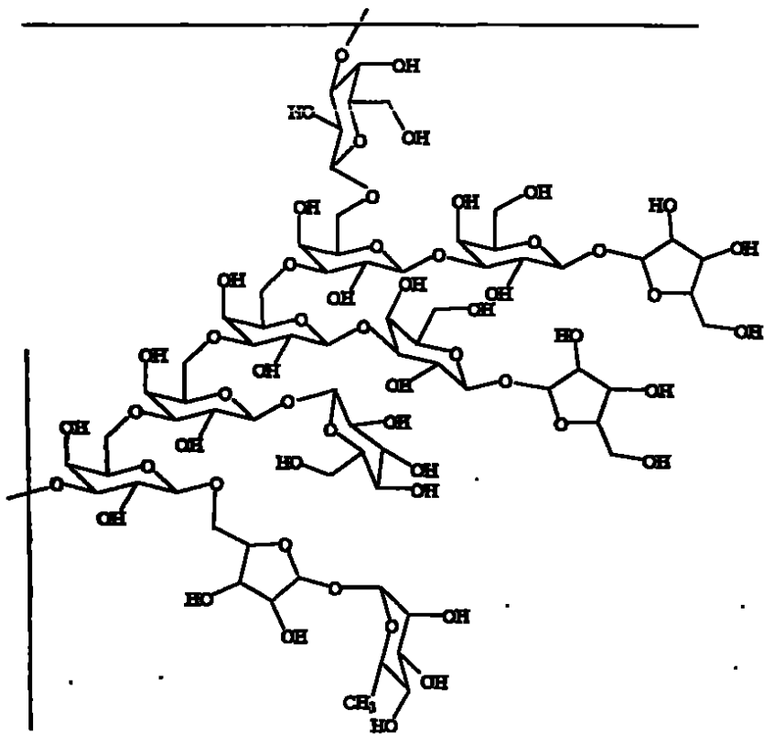

Fig. 2 : Proposed Structure of AGP Composition

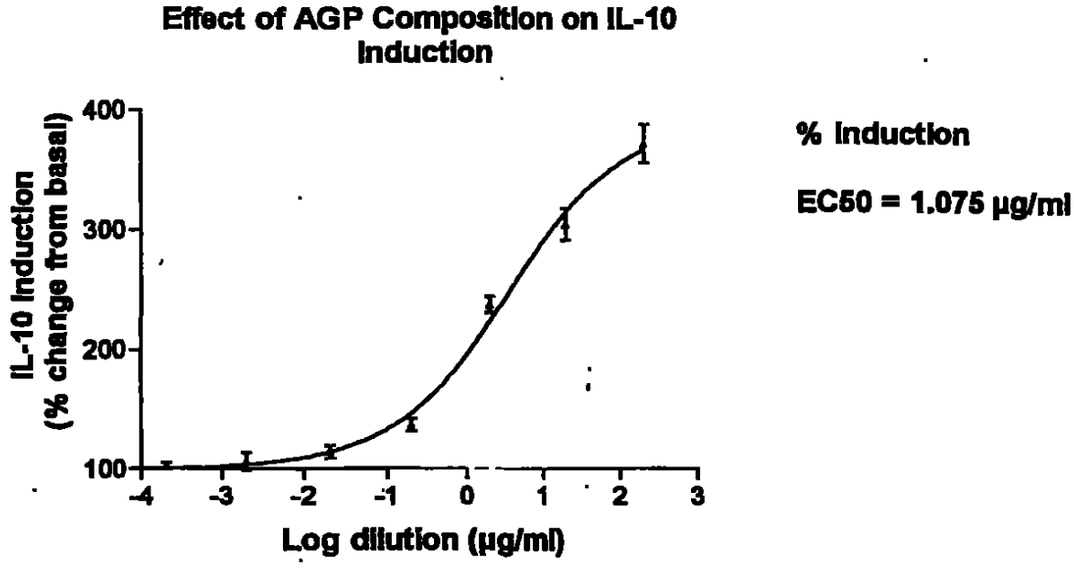

Fig. 3 : Effect of AGP Composition on IL 10 Induction

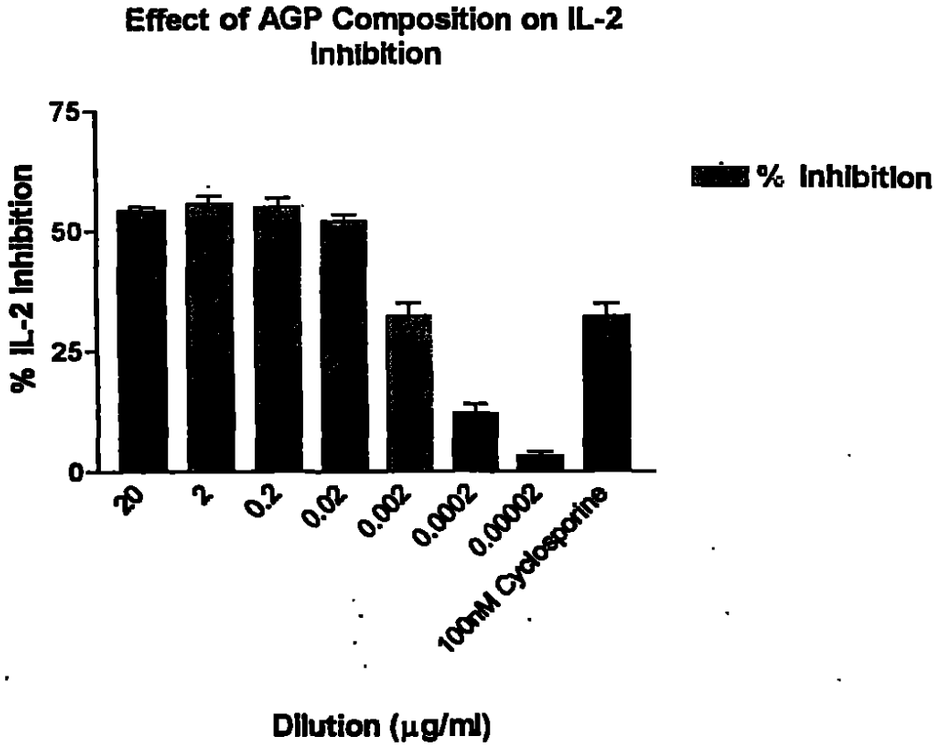

Fig. 4 : Effect of AGP Composition on IL-2 Inhibition

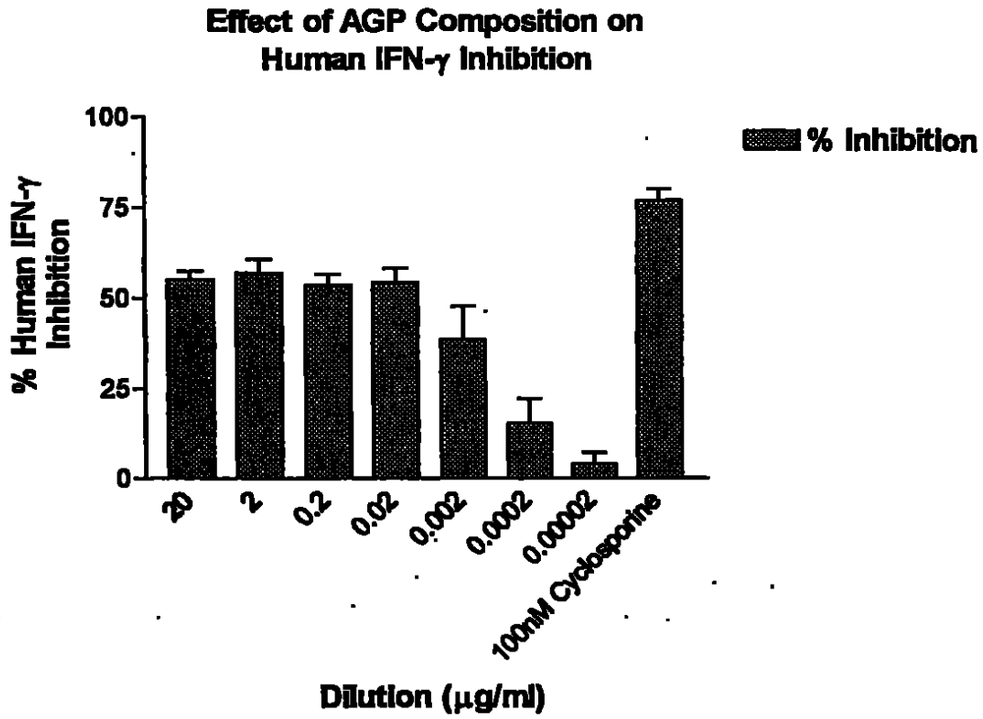

Fig. 5 : Effect of AGP Composition on Human IFN-γ Inhibition

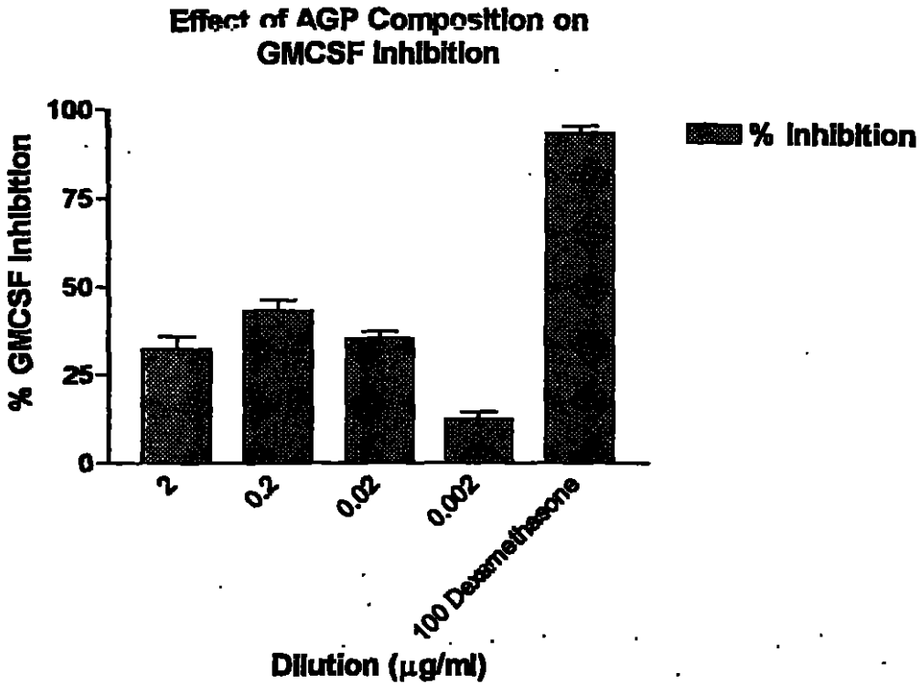

Fig. 6 : Effect of AGP Composition on GMCSF Inhibition

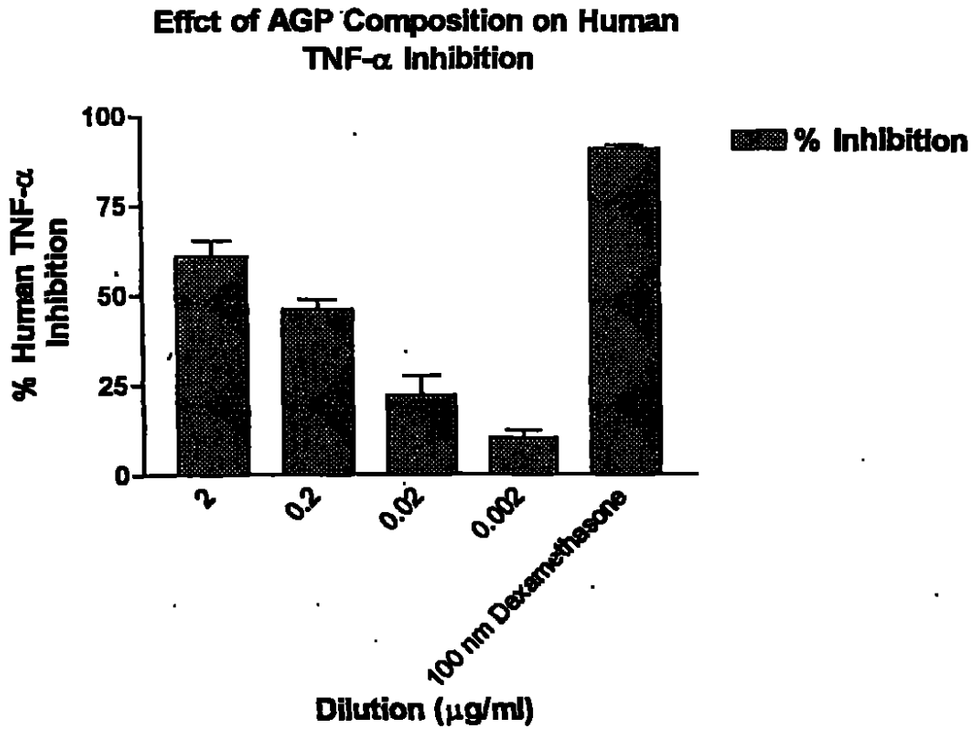

Fig. 6
7 : Effect of AGP Composition on Human TNF-α Inhibition

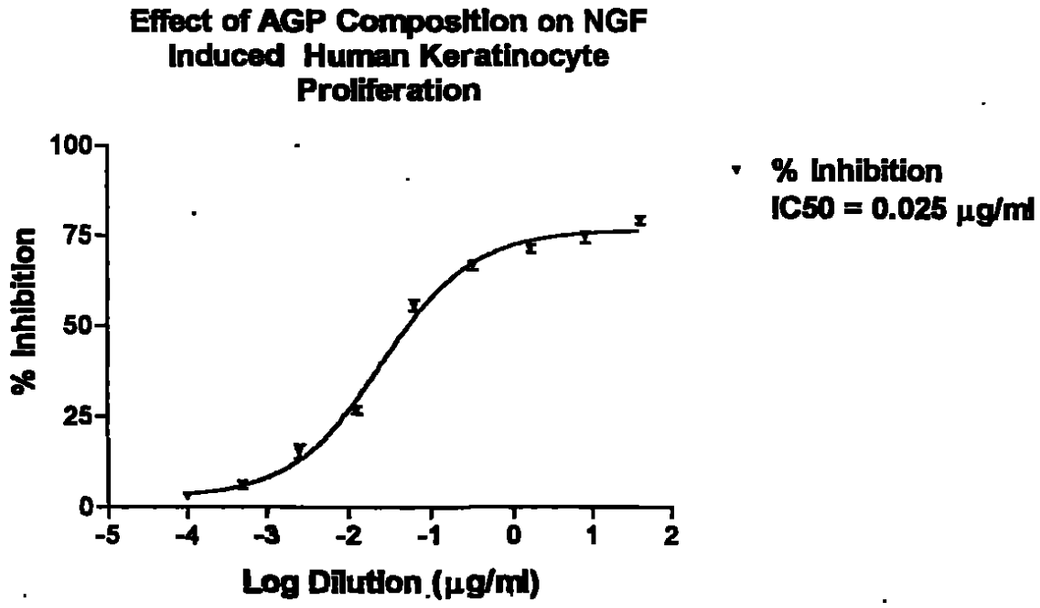

Fig. 8 : Effect of AGP Composition on NGF Induced Human Keratinocyte Proliferation

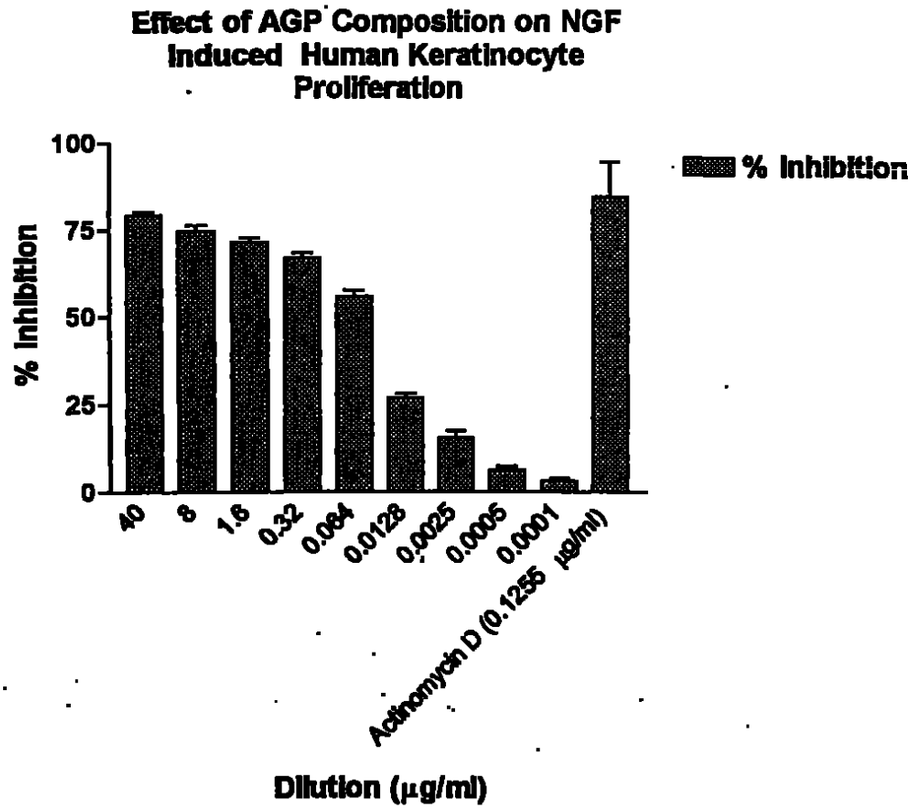

Fig. 9 : Effect of AGP Composition on NGF Induced Human Keratinocyte Proliferation

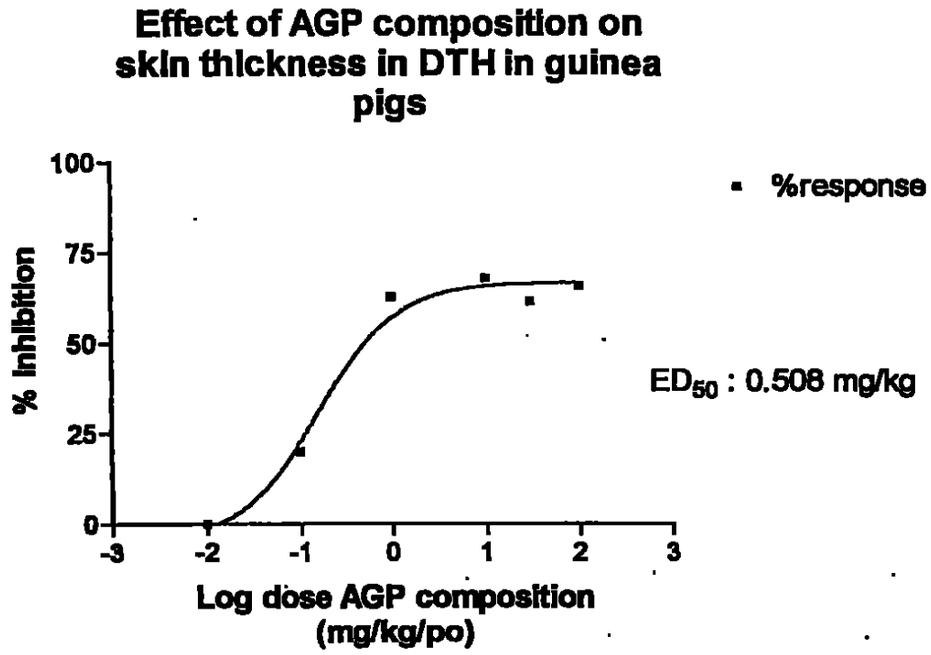

Fig. 10 : Effect of AGP Composition on Skin Thickness in PPD Challenged Guinea pigs

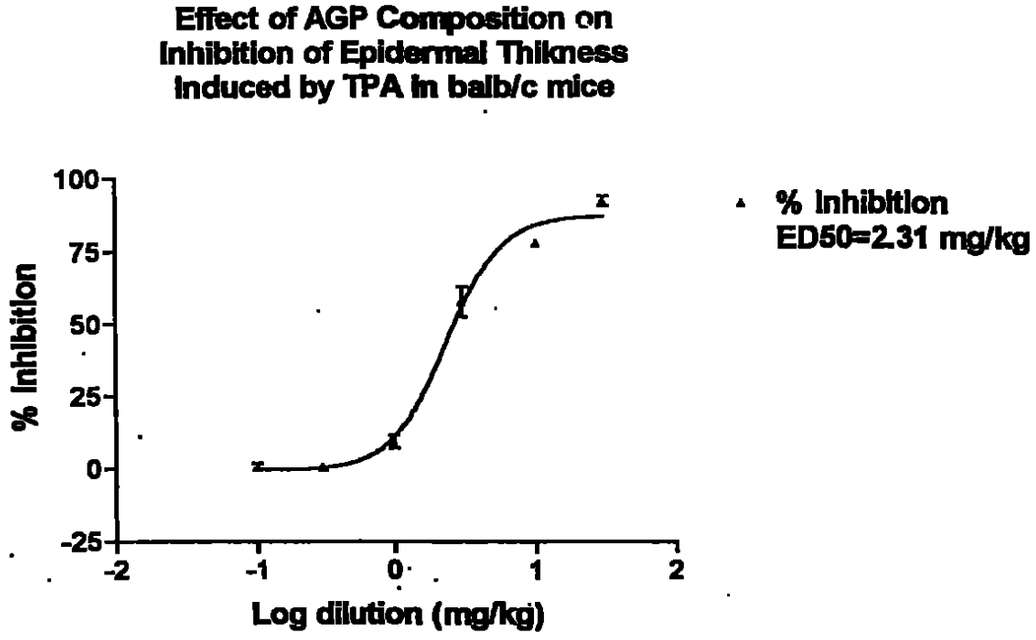

Fig. 11 : Effect of AGP Composition on Epidermal Thickness induced by TPA in balb/c mice

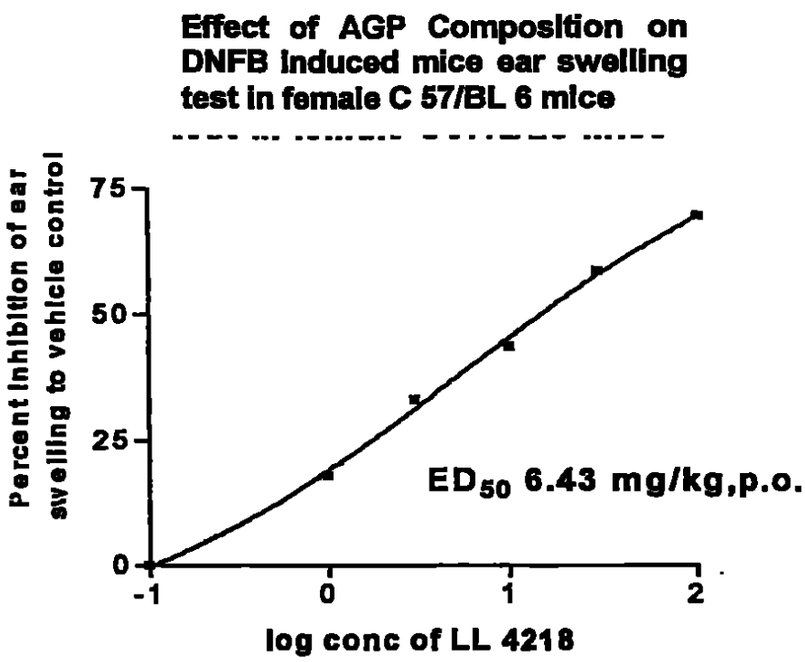

Fig. 12 : Effect of AGP Composition on DNFB Induced Mice Ear Swelling Test in Female C 57/BL 6 Mice

P.914

COMPOSICIÓN DE PROTEÍNA ARABINOGALACTÁN (AGP) PURIFICADA

CAMPO DE LA INVENCIÓN

La presente invención se relaciona con una composición de proteína arabinogalactán purificada (AGP) aislada a través de un método selectivo de las hojas y/o los tallos de la planta *Argemone mexicana*.

La presente invención también se relaciona con una composición de Proteína Arabinogalactán purificada (AGP) aislada de las hojas y/o los tallos de la planta *Argemone mexicana*, que tiene uno o más de los siguientes efectos: inmunosupresión, inhibición de la linfoproliferación, modulación de la citoquina tal como la inhibición de IL-2, inhibición del IFN-γ, o inducción del IL-10, inhibición de proliferación de queratinocito, actividad queratolítica y actividad inhibidora en ensayo de Hinchamiento de Oreja de Ratón (MEST).

DESCRIPCIÓN DE LAS ABREVIATURAS/NOTACIONES UTILIZADAS AQUÍ

Proteína Arabinogalactan:	AGP
Proteína Arabinogalactán de Alto Peso Molecular;	AGP-HM
Proteína Arabinogalactán de Bajo Peso Molecular:	AGP-LM.

BREVE DESCRIPCIÓN DE LAS FIGURAS

La Figura 1 es una gráfica que resume el proceso selectivo para aislar la composición AGP purificada.

La Figura 2 muestra la estructura propuesta de la composición AGP.

Las Figura 3 muestra el efecto de la composición AGP sobre la inducción de IL-10.

La Figura 4 muestra el efecto de la composición AGP sobre la inhibición de IL-2 porcentual.

La Figura 5 muestra el efecto de la composición AGP sobre la inhibición IFN-γ porcentual.

La Figura 6 muestra el efecto de la composición AGP sobre la inhibición del GMCSF.

La Figura 7 muestra el efecto de la composición AGP sobre la inhibición del TNFα humano.

La Figura 8 muestra el efecto de la composición AGP sobre la proliferación de Queratinocito Humano Inducido en NGF.

La Figura 9 muestra el efecto de la composición AGP sobre la proliferación del Queratinocito Humano Inducido NGF.

La Figura 10 muestra el efecto de la composición AGP sobre el Grosor de Piel el conejillo de indias inoculado PPD.

La Figura 11 muestra el efecto de la composición AGP sobre la inhibición del Grosor Epidérmico Inducido por TPA (12-O-tetradecanoilforbol-13-acetato) en ratones Balb/c.

La Figura 12 muestra el efecto de la composición AGP sobre el ensayo de hinchamiento de Oreja de Ratón Inducido por DNFB en Ratones Hembras C57/BL.

ANTECEDENTES DE LA INVENCIÓN

La psoriasis es un trastorno de la piel caracterizado por la hiperproliferación del queratinocito epidérmico inflamatorio y anormal que resulta en hiperplasia, engrosamiento de la epidermis y presencia de placas de escama roja. La condición crónica de la piel se reconoce por sus síntomas clínicos peculiares, caracterizados por parches rojos circunscritos cubiertos con escamas blancas que resultan en una piel escamosa y picazón. La psoriasis es una enfermedad muy visible y frecuentemente afecta la cara, cuero cabelludo, tronco y extremidades. La lesión en la enfermedad crónica típicamente está sometida a remisión y exacerbaciones.

Aunque, la psoriasis se manifiesta como un trastorno de la piel, mientras no se une a ninguna teoría, se cree que es una enfermedad de inmunidad mediada por célula dañada o defectuosa. En razón a que la aparición clínica de la psoriasis se origina en su mayoría por cambios epidérmicos, la enfermedad se ha considerado tradicionalmente como una de proliferación excesiva de queratinocito y diferenciación anormal. La evidencia habitual sugiere que los cambios epidérmicos en la psoriasis se originan por acciones de los linfocitos T en lesiones de la piel y que los linfocitos T inducen o sostienen el proceso de la enfermedad. La psoriasis se considera una enfermedad autoinmune, en donde los linfocitos T activados, que producen múltiples citoquinas originan anormalidades epiteliales secundarias. Los linfocitos desregulados producen citoquinas que estimulan la proliferación de los queratinocitos resistentes a la apoptosis. Las lesiones psoriáticas de la piel se caracterizan por inflamación, con las células T y los neutrófilos que infiltran tanto la dermis como la epidermis y la descamación excesiva relacionada con la hiperproliferación epidérmica y la diferenciación aberrante del queratinocito [Reich K., Garbe C., Blaschke V., Maurer C., Middel P., Westhal G., Lippert U., y Neumann *C., J. Invest. Dermatol.*, 2001, 116, 319].

Los trastornos autoinmunes son enfermedades originadas por el cuerpo que produce una respuesta inmune contra sus propios

tejidos. La causa de las enfermedades autoinmunes es desconocida, pero parece que existe una predisposición heredada en muchos casos en el desarrollo de una enfermedad autoinmune.

En unos pocos tipos de enfermedad autoinmune (tales como la fiebre reumática), una bacteria o virus dispara la respuesta inmune, y los anticuerpos o las células T atacan las células normales en razón a que ellas tienen algo de parte de su estructura que semejan una parte de la estructura del germen infeccioso.

Los trastornos autoinmunes caen en dos tipos generales: aquellos que dañan muchos órganos (enfermedades autoinmunes sistémicas), y aquellos donde un único órgano o tejido está directamente dañado por el proceso autoinmune (localizado). Algunos de los tipos más comunes de trastornos autoinmunes se resumen como sigue:

Enfermedades Autoinmunes Sistémicas	Enfermedades Autoinmunes localizadas
Artritis reumatoide (articulaciones, menos común pulmón, piel)	Diabetes mellitus tipo 1 (islotes del páncreas)
Lupus [Lupus Sistémico Eritematoso] (piel, articulaciones, riñón, corazón, cerebro, glóbulos rojos, otros)	Tiroiditis de Hashimoto, enfermedad de Graves (tiroides)

Escleroderma (piel, intestino, menos común pulmón)	Enfermedad Celiaca, enfermedad de Crohn, colitis Ulcerativa (tracto GI)
Síndrome de Sjogren (glándulas salivares, glándulas lacrimales, articulaciones)	Esclerosis Múltiple*, síndrome de Guillain Barré, (cerebro)
Síndrome de Goodpasture (pulmones, riñones).	Enfermedad de Addison (adrenal)
Granulomatosis de Wegener (senos, pulmones, riñones)	Esclerosis biliar primaria, colangitis Esclerosante hepatitis autoinmune (hígado)

El proceso inflamatorio involucra una serie de eventos que pueden ser elicitados por numerosos estímulos (por ejemplo, agentes infecciosos, isquemia, interacciones del antígeno-anticuerpo y lesiones térmicas y otras lesiones físicas). Cada tipo de estímulo provoca un patrón característico de respuesta que representa una variación relativamente menor sobre un tema. A un nivel macroscópico, la respuesta usualmente se acompaña por signos clínicos familiares de eritema, edema, sensibilidad a la presión y dolor.

Los síntomas observados en pacientes psoriáticos incluyen hiperplasia y cornificación anormal de las células epidérmicas atribuidas al cambio excesivo de las células por hipermetabolismo, astenia de respuesta inflamatoria en la capa epidérmica, vasodilatación y migración de leucocito e

infiltración en las capas de célula epidérmica. Sin embargo, se reconoce ahora que la hiperplasia epidérmica es una reacción a la activación del sistema inmune en regiones focales de la piel, que a su vez, es mediada por linfocitos T CD8+ y CD4+ que se acumulan en la piel enferma. De hecho, la psoriasis se reconoce ahora como la enfermedad inflamatoria mediada por célula T más prevalente en los humanos. Los síntomas de la psoriasis parecen así ser de excesivo rápido crecimiento de los queratinocitos y descamación de la superficie de la piel. En lesiones psoriáticas, el tiempo del ciclo de la célula de queratinocito se reduce aproximadamente 8 veces (36 vs. 311 horas en piel normal) y el número de células de división se duplica, resultando en una epidermis hiperplásica. La terapia con droga se dirige a retardar este proceso.

Se encuentra que la terapia PUVA agota los linfocitos de acuerdo con la mejora de la enfermedad. Estos datos son consistentes con un papel para las células T en patogénesis. Se encuentra que la Ciclosporina, un inmunosupresor conocido, tiene efectos dramáticos sobre la actividad de la enfermedad. En razón a que la ciclosporina tiene un efecto inhibidor mayor sobre la activación de la célula T, se inicia la formulación de argumentos de que la psoriasis es fundamentalmente una enfermedad inflamatoria.

Los linfocitos T deben infiltrar la dermis y luego adherirse a los queratinocitos para producir la placa psoriática. De esta

manera la adhesión de célula T regulante molecular y el tráfico se vuelven blancos terapéuticos mantenibles y su papel en patofisiología es de importancia considerable. Los eventos de adhesión intravascular se pueden inhibir al bloquear el disparo de quimioquina o bloquear la unión de integrina (LFA-1 A ICAM-1). El bloqueo de la integrina o la reducción de su expresión de superficie puede ser un evento importante para el tráfico de linfocitos que ayudan en la terapia anti-psoariática.

La inmunosupresión, la inhibición de linfoproliferación, modulación de citoquina tal como inhibición de IL-2, inhibición de IFN-γ, o inducción de IL-10; la inhibición de proliferación de queratinocito, la actividad queratolítica y la actividad inhibidora en MEST se conocen por estar involucradas en la actividad anti-psoriática.

El número de tratamientos diferentes y algunas veces tóxicos empleados para el mejoramiento de la psoriasis es testimonio de la naturaleza resistente de esta enfermedad. Como la mayoría (90%) de los pacientes con psoriasis tienen formas limitadas de la enfermedad, se pueden utilizar los tratamientos tópicos que incluyen ditranol, preparaciones de alquitrán, corticosteroides y los recientemente introducidos análogos de vitamina D3 (calcipotriol, calcitriol). Una minoría (10%) de los pacientes

con psoriasis tienen una condición más seria, por la cual un número de modalidades terapéuticas sistémicas están disponibles. Las terapias sistémicas específicas incluyen UVB, PUVA, metotrexato, derivados de vitamina A, (acitretin) e inmunosupresores tales como la ciclosporina A. La efectividad de la ciclosporina y el FK-506 para tratar la psoriasis suministran soportes para la hipótesis de la Célula T como la causa primaria de la enfermedad.

El uso tópico de corticosteroides reduce los síntomas de la psoriasis. Sin embargo su administración durante un largo periodo de tiempo, que es necesario en tal tratamiento origina taquifilaxis de tal forma que la dosis tiene que ser incrementada o se tienen que utilizar drogas más fuertes que conducen a la atrofia y acromasia o pérdida de pigmentación de la piel normal periférica, cuando esta se aplica tópicamente sobre la lesión psoriática [*British National Formulary* (BNF), Marzo 2001, No, 41].

El uso de fototerapia (irradiación con radiación ultravioleta) o fotoquimioterapia, que consiste de administración interna o externa de psoralenos y la aplicación de rayos ultravioleta de onda larga a la parte afectada, se asocia con desventajas como la posibilidad de envejecimiento acelerado o pigmentación de la piel

y de inducir carcinogenésis [*British National Formulary* (BNF), Marzo 2001, No, 41].

El uso externo de alquitrán de carbón, aunque se asocia con más pocos efectos colaterales cuando se comparan con los esteroides, es, sin embargo, desordenado y las desventajas incluyen olor fuerte, manchado de la piel etc. Ocasionalmente esto puede causar dermatitis estimulante.

El metotrexato, aunque es la droga de selección para tratar las condiciones psoriáticas, necesita ser cercanamente monitoreado por que este puede causar daño del hígado y/o disminuir la producción de oxigeno que llevan los glóbulos rojos, los glóbulos blancos que luchan contra la infección y las plaquetas que mejoran la coagulación. El uso a largo plazo de psoralenos y metotrexato incrementa significativamente el riesgo del carcinoma de célula escamosa en pacientes con psoriasis. [Stern R. S., and Laird N., *Cancer*, 1994, 73, 2759].

Los retinoides tales como etretinato se toman internamente por pacientes que sufren de psoriasis intractable: sin embargo esta es teratogénica y probable de acumular en el cuerpo por un largo periodo de tiempo y de esta manera se contraindica en caso de embarazo [Stern R. S., and Laird N., *Cancer*, 1994, 73, 2759].

El uso de agentes inumunosupresores macrocíclicos tales como la Ciclosporina, Tacrolimus y Ascomicina pueden dañar la función del riñón o causar hipertensión. Los posibles efectos colaterales de la hidroxiurea incluyen la anemia y una disminución en los glóbulos blancos y las plaquetas.

El Calcipotriol, un análogo de vitamina D3 sintético se ha vuelto uno de los tratamientos más ampliamente prescritos para la psoriasis. Sin embargo, este causa significativamente más irritación de la piel que los corticosteroides tópicos corrientes. Los efectos adversos comunes incluyen lesiones o irritación perilesional, irritación facial o del cuero cabelludo, o exacerbación de la psoriasis [Ashcroft D. M., Wan Po A. L. Williams H.C. y Griffiths C.E.M., *BMJ*, 2000, 320, 963]

Las aproximaciones biotecnológicas corrientes a los tratamientos de la psoriasis se relacionan con un ataque directo mediado por farmacéutico, bien sea sobre la proliferación de la célula o sobre el componente inmune de la enfermedad. Los inmunobiológicos inmunosupresores tales como el Clenoliximab, MEDI-507, ICM3, IDEC-114, SMART anti-CD3, Zenapax, Amavive, Hul 134, Xanelim, HuMaxCD4, IC747, IDEC-114 IDEC-131, Nuvion, DAB389IL-2, ONTAK y Etarnercept, conocidos por bloquear las respuestas inmunes en varias etapas están habitualmente bajo fases diferentes de ensayos clínicos.

Ninguno de los tratamientos anteriormente mencionados es, sin embargo, universalmente seguro y efectivo. La magnitud del impacto de la psoriasis es similar a aquella de otras enfermedades como la depresión, la hipertensión y la falla cardiaca congestiva. El costo del tratamiento de la enfermedad promedia los 800 dólares americanos por paciente al año en los Estados Unidos, y la enfermedad puede causar pérdida significativa de la productividad [Feldman S.R., *American Academy of Dermatology*, Agosto 2000].

Además, la enfermedad debido a su curso esporádico da una respuesta variable a los tratamientos, que también pueden tener efectos adversos. De esta manera, es una enfermedad difícil de curar. La naturaleza devastadora de la psoriasis se enfatiza por el alcance de los efectos colaterales que sufren los que poseen la enfermedad que están deseosos de alivio para lograr una remisión a una enfermedad que ellos saben que recurrirá más pronto o más tarde.

Además, aparte de las manifestaciones clínicas y la inconveniencia, el impacto psicológico de la enfermedad sobre la vida de los pacientes es tremendo. La psoriasis es una condición compleja que afecta todos los aspectos de la emoción y el debilitamiento físico del paciente y, reduce sustancialmente la

calidad de vida de millones de personas en todo el mundo. Más aún, como es a menudo claramente visible, los individuos afectados sufren enfermedades marcadas, apenamiento e incomodidad [Fortune D.G., Richards H. L., Main C. J., and Griffiths C.E. M. *Am. Acad. Dermatol.*, 1998, 39, 196]

Una composición derivada de una fuente vegetal, que suministra un tratamiento seguro, bien tolerado y efectivo de la psoriasis y que más aún soluciona los inconvenientes y las limitaciones de los tratamientos habituales se ha descrito en nuestra Publicación de Patente de los Estados Unidos No. 2003/0194456 A1.

La publicación de Patente Estadounidense No. 2003/0194456 A1 describe actividades inmunológicas y farmacéuticas *in vitro* e *in vivo* útiles de una medicación/composición que comprende un extracto obtenido de las hojas o tallos de la planta, *Argemone mexicana*, opcionalmente en combinación con un extracto obtenido de los frutos de la planta, *Cuminum cyminum* para el tratamiento y la profilaxis de la psoriasis y otros trastornos. El extracto que puede ser un extracto acuoso, etanólico o acuoso-etanólico, aparte de exhibir actividades inmunológicas y farmacológicas útiles suministra la reducción significativa de la proporción del área de psoriasis y la Calificación del Índice de Severidad (PASI) con mejor tolerabilidad dentro del rango de límites normales permisibles. En prueba de los estudios de concepto

conducidos sobre pacientes que tienen psoriasis tipo placa crónica, una composición que comprende el extracto anteriormente mencionado cuando se administra oralmente se encuentra que resulta en la reducción de la calificación del PASI de 6.33 ± 2.84 a 0.90 ± 1.27, con un estado libre de enfermedad observado en algunos pacientes después de 8 semanas de tratamiento.

La Publicación de Patente Estadounidense No. 2003/0194456 A1 reporta además la toxicidad aguda (LD_{50}) del extracto obtenido de las hojas y/o tallos de la planta *Argemone mexicana*, evaluada en ratones y ratas a través de las rutas oral i.v. de administración por ser > 1000 mg/kg de peso corporal del animal con 50% de mortalidad.

Se puede mencionar aquí que la planta *Argemone mexicana*, se compone de varios compuestos, que incluyen *inter alia*:

i) alcaloides tales como protopina, nitrato de protopina, berberina, nitrato de berberina, criptopina, alocriptopina, coptisina, sanguinarina, dihidrosanguinarina, norsanguinarina, 6-acetonil dihidrosanguinarina, dihidroqueleritrina, queleritrina, norquelelitrina, 6-acetonil dihidroqueleritrina, (-) queilantifolina, (-) β escoulerina, metohidróxido (-) -α

estilopina (-)-α- y β-estilopina metohidróxidos, (-) queilantifolina, 6-acetonil dihidrosanguinarina, metohidróxido de (-)α-tetrahidropalmatina, reticulita, talifolina, muramina, argemonina, norargeminina, argemexicaina A, argemexicaina B, N-demetiloxisanguinarina; (+)-1,2,3,4-tetrahidro -1-(2-hidroximetil-3,4-dimetoxifenilmetil)-6,7-metilenodioxi-isoquinolina, heleritrina, y oxihidrastinina;

ii) flavonoides tales como isormanetina, isormanetin-3-glucósido; isorhamnetina, -3-O-glucósido, isorhamnetin-3,7-diglucósido; 3-metoxi quercetina, quercetin 5,3',4' trimetil éter; luteolina argemexitin y eriodictiol;

iii) Ácidos grasos, tales como palmítico, esteárico, araquídico, oleico, linoleico, laúrico, behénico, lignocérico, hexadecenoico, ricinoleico, 11-oxo-triacontanoico y 11-hidroxi triacontanoico;

iv) Aminoácidos, tales como histidina, lisina, ácido glutámico, glicina, alanina, leucina, valina, fenil alanina, tirosina, treonina, arginina, cerina, asparagina, cisteína, metionina, triptofan, hidroxiprolina, prolina, L-glutamina, hidroxiprolina, β-alanina, y ácido aspártico;

v) Carbohidratos, tales como glucosa y fructosa y glicósidos;

vi) Ácidos orgánicos, tales como succínico, cítrico, tartárico, maleico, y málico;

vii) Otros compuestos como ceril alcohol β-sitosterol, nitrato de potasio, fosfato de calcio y sulfato de calcio.

Se ha encontrado que el extracto obtenido de las hojas y/o tallos de la planta *Argemone mexicana* que utiliza el proceso para la extracción por ejemplo maceración y percolación, utilizando agua, etanol y mezclas de éstos descrito en la Publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 contiene sustancialmente todos los compuestos anteriormente mencionados. En otras palabras, el extracto se compone de todos los compuestos presentes en las partes de las plantas utilizados para la extracción es decir, se compone de una mezcla de alcaloides, flavonoides, ácidos grasos, ácidos orgánicos, aminoácidos, azúcares y sales.

Los extractos, así obtenidos por el proceso descrito en la Publicación de la Solicitud de Patente Estadounidense No.

2003/0194456 A1 se encuentra que exhiben actividades inmunológicas y farmacológicas *in vitro* e *in vivo* por ejemplo, inmunosupresión, inhibición de la linfoproliferación, modulación de la citoquina tal como la inhibición de IL-2, inhibición de IFNγ, e inducción del IL-10, inhibición de la proliferación de queratinocito, actividad queratolítica, inhibición de proliferación de célula endotelial, inhibición de expresión de molécula de adhesión celular tal como ICAM-1, inhibición del MEST, e inhibición de las enzimas tales como la quinasa Tirosina p60src, que se conocen por estar involucradas en actividad anti-psoriática.

Además, la Publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 enseña que los extractos anteriormente mencionados de las hojas y/o los tallos de la planta *Argemone mexicana* pueden ser fraccionados utilizando solventes alcohólicos tales como n-butanol y metanol y las fracciones obtenidas de estas también exhiben actividades inmunológicas y farmacológicas *in vitro* e *in vivo* que incluyen actividad anti-psoriática.

El procedimiento de fraccionamiento de los extractos acuosos de las hojas y/o tallos de la planta *Argemone mexicana* descrito en la Publicación de la Solicitud de Patente No. 2003/0194456 A1 se logra a través de cromatografía de partición líquido - líquido

multietapa, precipitación y secado de los extractos y suministra fracciones que contienen sustancialmente diferentes clases de compuestos como componentes mayores.

Por ejemplo, de acuerdo con el método descrito en la Publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 una fracción soluble en n-butanol se prepara al agregar n-butanol al extracto acuoso de las hojas y/o tallos de la planta *Argemone mexicana*, separación de la capa n-butanol de la fase acuosa, seguido por el lavado de la capa n-butanol con agua y la evaporación del solvente bajo presión reducida para dar la fracción soluble de n-butanol como una masa viscosa. La capa acuosa, se mezcla con metanol, en donde se efectúa la precipitación de la masa sólida. La masa sólida se separa, y la masa disuelta en agua se liofiliza para dar la fracción insoluble en metanol. El filtrado obtenido después de la separación del precipitado insoluble en metanol de la mezcla de metanol-agua sobre la evaporación da la fracción soluble de metanol como una masa sólida.

Típicamente, la planta *Argemone mexicana* produce aproximadamente 3-4.5% de fracción soluble de n-butanol; 46-54% de fracción soluble en metanol, que tiene un número base total entre 290-340; y 24-30% de fracción insoluble en metanol, que tiene un número base total entre 350-380. Como se utiliza aquí el número base es

la cantidad de ácido que se requiere para neutralizar todos los constituyentes básicos presentes en 1 g de la muestra.

Como se mencionó anteriormente, las tres fracciones difieren sustancialmente en la constitución de los compuestos contenidos allí. La fracción soluble en n-butanol se encuentra que contiene alcaloides, flavonoides y otros compuestos de bajo peso molecular; la fracción soluble en metanol se encuentra que contiene aminoácidos, ácido orgánico y sales, mientras que la fracción insoluble en metanol se encuentra que contiene azúcares, ácidos orgánicos y sales.

Aunque, las investigaciones químicas extensivas durante los años sobre diferentes partes de la planta *Argemone mexicana* han resultado en el aislamiento de un número de alcaloides, flavonoides, aminoácidos, ácidos orgánicos, ácidos grasos etc., sin embargo, no se ha conducido ni reportado ningún estudio sistemático para el aislamiento e identificación de otros principios presentes en la planta.

Uno de tales principios, comúnmente encontrados en el reino vegetal son las proteínas de Arabinogalactán (AGP), que son esencialmente macromoléculas de polisacáridos en los cuales el carbohidrato está asociado con o ligado a proteínas. La AGP se compone principalmente de residuos de arabinosa y galactosa.

Estos ocurren en plantas como polisacáridos en asocio con cantidades variantes de proteínas, y generalmente contienen una alta proporción de carbohidratos con comparativamente menos proporción de proteínas, usualmente menos de 10 proteínas, aunque, se conocen los AGP que tienen contenidos superiores de proteínas, también los AGP son ampliamente distribuidos en la mayoría de las plantas superiores tales como la *Echinacea purpurea, Nicotiana alata, Vitis vinifera, Diospyros kaki, Gladiolus gandavensis, Lolium multiflorum*, [Anderson, R. L. Clarke, A. E., Jermin, M.A. Knox, R.B. and Stone, B.A., Australian *J. Plant. Physiol.*, *1977*, 4, 143-158], *Phaseolus vulgaris* [Hawes, g.b., Adams, G.A., *Phytochemistry*, **1972**, 11, 1461-1465] y *Acacia arabica* [Classen, B., Witthohn, K, and Blaschek, W., *Carbohidrate Research*, **2000**, 327, 497-504].

Las AGP poseen propiedades de soporte adhesivo y de agua y responden a heridas e infecciones en las plantas, Estas determinan la identidad celular y las interacciones específicas. Estas también juegan un papel en la diferenciación celular y de tejido así como también en controlar la embriogénesis somática. Ellas también se valoran por las varias actividades biológicas [*WO 01/00682 A1*, **2001**] Existen dos tipos de AGP, el AGP I y el AGP II. El último es decir el AGP II contiene un núcleo de galactosa y está altamente ramificado, contiene usualmente menos de 10% de proteína y posee cadenas laterales altamente

sustituidas por residuos de arabinofuranosilo y algunas veces otros azúcares como la ramnosa, glucosa, manosa, etc. También se reporta la presencia de ácidos urónicos y derivados sustituidos.

Como se mencionó anteriormente, aunque, las investigaciones químicas sobre todas las partes de la *Argemone mexicana* han sido conducidas, sin embargo, ningún estudio ha estado dirigido hacia el aislamiento, caracterización y entendimiento de las propiedades biológicas de los AGP presentes en ciertas partes de la planta.

RESUMEN DE LA INVENCIÓN

La presente invención suministra un método selectivo para el aislamiento de los AGP provenientes del extracto obtenido de las hojas y/o tallos de la planta, *Argemone mexicana* en forma purificada, que exhibe actividad anti-psoriática bastamente superior y otras actividades inmunológicas y farmacológicas útiles comparadas con el extracto y las fracciones de las hojas y/o tallos de la planta, *Argemone mexicana,* como se describió en la Publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1. En particular, la actividad anti-psoriática bastamente superior exhibida por los AGP purificados obtenidos mediante el método selectivo se encuentran que son altamente útiles en la preparación de una composición farmacéutica que

comprende los mismos y suministra de esta manera un tratamiento seguro efectivo y bien tolerado y forma la base de la presente invención.

Por lo tanto, un aspecto de la presente invención es suministrar un método selectivo para el aislamiento de la Composición, de proteína de Arabinogalactán (AGP) en forma altamente pura de las hojas y/o tallos de la planta *Argemone mexicana*.

En otro aspecto, la presente invención suministra una composición de proteína de Arabinogalactam purificada (AGP), que tiene un rango de peso molecular promedio entre 10 KD a 150 KD, aislado de las hojas y/o tallos de la planta *Argemone mexicana*.

Otro aspecto de la presente invención es suministrar una composición anti-psoriática y el tratamiento para la psoriasis, obtenido de las hojas y/o tallos de la planta *Argemone mexicana*, que no solamente es altamente segura, efectiva y bien tolerada sino más aún, soluciona los inconvenientes y limitaciones del régimen terapéutico habitual.

Otro aspecto de la presente invención es suministrar una composición de proteína Arabinogalactán (AGP) purificada aislada a través de un método selectivo de las hojas y/o tallo de la planta *Argemone mexicana*, que exhibe propiedades inmunológicas y

farmacológicas útiles tales como una o más de inmunosupresión, inhibición de linfoproliferación, modulación de citoquina tal como inhibición de IL-2, inhibición de IFN-γ, o inducción del IL-10, inhibición de la proliferación de queratinocito, actividad queratolítica, e inhibición del MEST.

Aún otro aspecto adicional de la presente invención es suministrar una composición de proteína Arabinogalactán (AGP) purificada. Aislada a través de un método selectivo de las hojas y/o tallos de la planta *Argemone mexicana* para el tratamiento o profilaxis de uno o más de los trastornos tales como dermatitis, escleroderma, eccema; trastornos inflamatorios y otras enfermedades autoinmunes como la artritis psoriática, artritis reumatoide, enfermedad de Cronh, esclerosis múltiple, enfermedad del intestino irritable, espondilitis anquilosante, lupus sistémico eritematoso y síndrome de Sjogren; y/o alergias como el asma y la enfermedad pulmonar obstructiva crónica.

Aún otro aspecto de la presente invención es suministrar una composición farmacéutica que se puede utilizar para el tratamiento y/o la profilaxis de las enfermedades y condiciones anteriormente mencionadas que comprenden la composición de la proteína Arabinogalactán purificada (AGP) aislada a través de un

método selectivo de las hojas y/o tallos de la planta *Argemine mexicana*.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

A menos que se defina otra cosa, todos los términos técnicos y científicos tienen el significado como se entiende comúnmente por un experto en la técnica al cual pertenece la invención.

Como se utiliza aquí la frase "composición de proteína de Arabinogalactan (AGP) purificada" significa una composición que consiste esencialmente de solamente proteínas Arabinogalactan que pueden incluir cantidades en trazas de otros componentes obtenidos de la extracción del tallo y/o las hojas de la planta *Argemone mexicana* de acuerdo a esta invención. La composición de AGP purificado se distingue de la composición farmacéutica que comprende la composición AGP por que la composición AGP purificada no incluye ningún excipiente farmacéuticamente aceptable.

En nuestra publicación de Solicitud de Patente Estadounidense No. 2003/0194456 A1 se describe un proceso para obtener un extracto de las hojas y/o los tallos de de la planta *Argemone mexicana* y el fraccionamiento del extracto con n-butanol y metanol para dar

las respectivas fracciones solubles en n-butanol, soluble en metanol e insoluble en metanol.

El método para la preparación de las varias fracciones del tallo y/o las hojas de la planta *Argemone mexicana* se describe en la publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 comprende las etapas de:

i) preparación de un extracto del tallo y/o las hojas de la planta *Argemone mexicana* a través de un número de procedimientos de extracción, sin embargo, preferiblemente a través de la maceración multietapa, sucesiva y la perforación utilizando solventes tales como agua, etanol o mezclas de éstos a temperatura ambiente para obtener los extractos correspondientes que están constituidos de alcaloides, flavonoides, aminoácidos, ácidos orgánicos, azúcares y sales. Estos extractos se pueden utilizar como tal o son sometidos a liofilización para dar una masa liofilizada, las cuales ambas fueron utilizadas para fraccionamiento;

ii) particionar el extracto como se obtuvo o una solución de la masa liofilizada en agua como se obtuvo en la etapa i) con n-butanol, y la separación de a capa de n-butanol de la fase acuosa seguido por lavado de la capa de n-butanol con agua y la evaporación del solvente bajo presión reducida para dar una masa viscosa de la fracción soluble de n-butanol, que está constituida

principalmente por alcaloides, flavonoides, y otros compuestos de bajo peso molecular;

iii) La mezcla de agitación de la capa acuosa de la tapa ii) con aproximadamente 6 a 7 veces su volumen de metanol y filtración/centrifugación de los sólidos precipitados y el secado para dar la fracción insoluble en metanol, que está constituida primariamente de polisacáridos, ácidos orgánicos y sales. Este material se disolvió en agua y se liofilizó para dar un polvo liofilizado;

iv) concentración del filtrado es decir la solución metabólica acuosa proveniente de la etapa iii) bajo presión reducida para dar una masa sólida de la fracción soluble en metanol, que está constituida primariamente de aminoácidos, ácidos orgánicos y sales inorgánicas.

La fracción insoluble en metanol preparada de acuerdo con el método descrito en la publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 se aisló en un rendimiento de aproximadamente el 33%. Aunque existe mención en la especificación de que la fracción está constituida de azúcares, ácidos orgánicos y sales, sin embargo, estos constituyentes no fueron caracterizados en ese momento.

Las presentes investigaciones revelan que la reacción insoluble en metanol obtenida como por el método descrito en la publicación

de Solicitud de Patente Estadounidense No. 2003/0194456 A1 está constituida de aproximadamente el 3% en peso de los AGP, (con respecto al extracto acuoso), el resto de los constituyentes son ácidos orgánicos, aminoácidos y sales inorgánicas, es decir, la fracción insoluble en metanol obtenida de acuerdo con el método descrito en la publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1.está constituida de los AGP en mezcla con otros componentes. La fracción insoluble en metanol contiene los AGP, aunque en baja concentración y en una forma altamente impura.

Los presentes inventores han encontrado que los AGP se pueden aislar y purificar en una forma purificada en un rendimiento de aproximadamente 0.06% de rendimiento con respecto a los tallos y/o las hojas de *Argemone mexicana* o aproximadamente 1% con respecto al extracto acuoso liofilizado o preparado de esta a través de un método altamente selectivo y que los AGP aislados exhibieron una actividad anti-psoriática bastamente superior sobre aquellas obtenidas a través del método descrito en la publicación de Solicitud de Patente Estadounidense No. 2003/0194456 A1.

El método selectivo para el aislamiento de los AGP en forma purificada de las hojas y/o tallos de la planta *Argemone mexicana* comprende las etapas de:

a) La extracción de una parte en peso de las hojas y/o los tallos de la planta *Argemone mexicana* con 1 a 10 partes en peso de agua, un alcohol C_1-C_3 o una mezcla de estos para obtener un extracto acuoso que puede ser pero no tiene que ser parcial o completamente concentrado o liofilizado;

b) Las remoción de los componentes ácidos y básicos del extracto acuoso, extracto parcialmente concentrado, una solución acuosa del extracto completamente concentrado o el extracto liofilizado obtenido en la etapa a) al someter que el extracto parcialmente concentrado o la solución acuosa del extracto concentrado a la cromatografía de intercambio de ión para obtener un extracto acuoso neutro;

c) fraccionamiento del extracto acuoso neutro obtenido en la etapa b) con n-butanol para dar la fracción soluble en n-butanol;

d) mezcla y agitación de los lavados acuosos de la etapa c) con metanol o etanol y aislamiento de los sólidos precipitados para obtener la fracción insoluble en metanol o etanol; y

e) someter el metanol o la fracción insoluble en etanol obtenida en la etapa d) a cromatografía de gel y cromatografía de

exclusión de tamaño en sucesión para obtener una proteína de Arabinogalactán purificada (AGP).

El método selectivo de aislamiento del AGP purificado se resume en La Figura 1.

Para la extracción, se pueden utilizar las hojas, tallos o ambos de la planta *Argemone mexicana*. Preferiblemente las hojas frescas, tallos o ambos son utilizados, y estos son molidos hasta una textura de pasta fina o antes de la extracción.

En una modalidad de la invención, una parte en peso de las hojas y/o tallos molidos de *Argemone mexicana* son extraídos con 1 a 10 partes en peso de agua. El alcohol C_{1-3}, una mezcla de estos 2 a 4 veces y los extractos combinados percolados durante 2 a 20 horas a una temperatura de entre 20EC a 45EC.

En otra modalidad, una parte en peso de las hojas y/o tallos molidos frescos de la *Argemone mexicana* son extraídos con 1 a 3 partes de agua, alcohol C_{1-3}, o una mezcla de 2 a 4 veces los extractos combinados percolados durante 2 a 16 horas a una temperatura entre 20EC a 45EC.

En otra modalidad, una parte en peso de las hojas y/o tallos molidos frescos de *Argemone mexicana* son extraídos con 1 a 1,5

partes en peso de agua, un alcohol C_{1-3}, o una mezcla de agua y el alcohol C_{1-3}, de estos 2 a 4 veces con los extractos combinados percolados durante 16 horas a temperatura ambiente.

El alcohol C_{1-3}, se selecciona de metanol, etanol, 1-propanol, y 2-propanol, preferiblemente etanol.

Después de la percolación, el extracto es filtrado centrifugado y el filtrado se puede concentrar parcialmente a un cierto volumen del volumen original del extracto o se puede concentrar hasta sequedad o se puede liofilizar.

Cuando el extracto es parcialmente concentrado este se concentra a un volumen entre 1/5 a 1/10 del volumen original del extracto. Cuando el extracto original se concentra hasta sequedad p se liofiliza, el extracto seco o el polvo liofilizado se pueden redisolver en dosis 50 veces en peso de agua a una parte en peso de la masa seca/liofilizada antes de la cromatografía de intercambio de ión.

En una modalidad preferida el extracto acuoso liofilizado se disolvió en 12 veces agua antes de la cromatografía de intercambio de ión.

Bien sea la solución del extracto parcialmente concentrado o la solución acuosa del concentrado liofilizado obtenido sobre la concentración completa del extracto original es entonces sometida a cromatografía de intercambio de ión secuencial sobre una resina de intercambio de catión seguido por cromatografía sobre una resina de intercambio de anión o en otra modalidad cromatografía de intercambio de ión sobre una resina de intercambio de anión seguida por cromatografía sobre resina de intercambio de catión para lograr un extracto acuoso neutro.

Las resinas de intercambio de catión y anión se emplean en proporciones de una parte a 50 partes en peso al volumen del extracto utilizado.

Las resinas de intercambio de catión adecuadas se pueden emplear incluyendo intercambiadores de catión de ácido fuerte de polietileno sulfonatado e intercambiadores de catión de ácido débil tipo ácido carboxílico. Las resinas intercambiadoras de catión adecuadas incluyen pero no están limitadas a intercambiadores de catión de ácido fuerte de poliestireno sulfonatado comercialmente disponibles y comúnmente utilizadas como AG 50W (Biol-Ral, USA), Amberlite IR-20(Rohm and Hass, USA), Dowex 50W (Dow Chemical Co., USA), Duolite 225 (Día-Prosim Ltd), Permutit RS (Permutit AG, Germany), and Permutite C50D (Philips and Pain-Vermorel, France); e intercambiadores de catión de ácido

débil tipo ácido carboxílico como Amberlite IRC-50 (Rohm and Haas, USA), Bio-Rex 70 (Bio-Rad, USA), Chelax 100 (Bio-Rad, USA), Duolite 436 (Día-Prosim Ltd), Permutit C (Permutit AG, Alemania), y Permutit H y H-70 (Permutit Co., USA).

Se pueden emplear resinas intercambiadoras de anión adecuadas que incluyen intercambiadores de anión de base débil tipo amina alifática e intercambiadores de anión de base fuerte. Las resinas intercambiadoras de anión incluyen pero no están limitadas a los intercambiadores de anión de base débil tipo amina alifática comercialmente disponibles y comúnmente utilizadas. Como Amberlite IR-45 e IRA-67 (Rohm and Hass, USA), Dowex 3 (Dow Chemical Co., USA), Permutite E (Permutit AG, Germany), Permutit A 240A (Philips and Pain-Vermorel, France); y en intercambiadores de unión de base fuerte como AG 2x8, (Bio-Rad, USA), Amberlite IRA-400 (Rohm and Haas, USA), Dowex 2x8 (Dow Chemical Co., USA), Duolite 113 (Día-Prosim Ltd), Permutit ESB (Permutit AG, Alemania), y Permutite 330D (Philips and pain-Vermorel, France).

El extracto acuoso neutro así obtenido se puede liofilizar y la masa liofilizada se puede particionar entre n-butanol y agua para la siguiente etapa de fraccionamiento. En la alternativa, el extracto acuoso neutro se puede utilizar como tal para el fraccionamiento con n-butanol. Para la efectividad en costo es preferible utilizar la solución de fracción neutra obtenida

después de pasar a través de resinas intercambiadoras de catión y anión como tales para el fraccionamiento con n-butanol.

Típicamente, la masa liofilizada anteriormente mencionada del extracto neutro reparticiona en una mezcla de agua n-butanol por 1 parte de masa liofilizada. A la solución se le permite permanecer y la capa de n-butanol se separa de la fase acuosa. La etapa se repite 2 a 4 veces y las capas acuosas combinadas se utilizan para fraccionamiento adicional con metanol o etanol.

En un ejemplo de la invención 1 parte del volumen de la solución de fracción acuosa neutra obtenida después de pasar a través de las resinas de intercambio de catión (Amberlite IR 120) y anión (Amberlite IRA 400) se agrega a aproximadamente 10 partes en volumen de n-butanol. Las fases se mezclan, se les permite permanecer y la capa de n-butanol se separa de la fase acuosa. La etapa se repite 2 a 4 veces y las capas acuosas combinadas se utilizan para fraccionamiento adicional con metanol o etanol.

La fase acuosa combinada obtenida en la etapa anteriormente mencionada se mezcla con metanol o etanol y se agita para precipitar la fracción insoluble de metal/etanol. Típicamente, el metanol o etanol se emplea en proporciones de 1 a 20 veces el volumen por volumen de extracto acuoso.

El sólido precipitado, que contiene el AGP se aísla por medios convencionales, tal como decantación, filtración, centrifugación, etc. y luego se seca para producir un polvo amorfo café.

El sólido AGP así obtenido, que contiene AGP-HM y AGP-LM se somete adicionalmente a cromatografía de gel secuencial y a cromatografía de exclusión de tamaño para obtener AGP-HM y AGP-LM purificado.

La cromatografía de gel se lleva a cabo utilizando técnicas convencionales y absorbentes poliméricos tales como absorbentes de Amberlite XAD-2, XAD-4 o XAD-7, preferiblemente XAD-7. El AGP sólido e impuro se aplica en una banda estrecha a la parte superior de la columna requisito y se lava por medio de fase móvil que es agua, Las fracciones que contienen el AGP son recolectadas.

El eluído que contiene el AGP impuro se somete adicionalmente a cromatografía de exclusión de tamaño utilizando técnicas convencionales. El Sefacril (1:25-1:50) se puede utilizar para obtener AGP, AGO (HM) y AGP (LM) purificados de la presente invención. El sefacril S-100, S-200 HR Y S-300 HR comercialmente disponible se puede utilizar siendo el preferido el S-200 HR. El Sefacril se puede emplear en una proporción de 1 a 2 partes en

peso a 25 A 250 partes en volumen de la solución de AGP obtenida después de cromatografía de gel.

En una modalidad, el AGP crudo (100 mg) se disuelve en agua y se carga sobre Sefacril S-200(25 ml). La columna se eluye a una proporción de 0.5 ml/minuto y se recolectan 50 fracciones. Todas las fracciones son monitoreadas mediante HPLC-ELSD. Las fracciones 10 a 20 se combinaron sobre la base del HPLC para producir AGP-HM y las fracciones 25 a 35 también se combinaron para producir AGP-LM, los componentes de la composición AGP tienen un rango de peso molecular promedio entre 10 KD y 150 KD.

Luego de la cromatografía de exclusión de tamaño el AGP purificado de la presente invención se puede aislar de la solución acuosa a través del empleo de técnicas convencionales tales como evaporación, liofilización, secado por rociado, secado por congelamiento etc.

El AGP purificado, así obtenido se encuentra que muestra un rango de peso molecular promedio entre 10 KD a 150 KD. Se encuentran para exhibir uno o más de los siguientes efectos: inmunosupresión, inhibición de linfoproliferación, modulación de citoquina tal como inhibición IL-2, inhibición IFN-λ, Inducción IL-10, inhibición de la proliferación de queratinocito, actividad

queratolítica e inhibición MEST. Estos se conocen por estar involucrados en trastornos inflamatorios, enfermedades autoinmunes y alergias. Estos también se conocen por estar involucrados en actividad anti-psoriática, dermatitis, escleroderma, eczema y dermatitis atópica. Los trastornos inflamatorios y las enfermedades autoinmunes incluyen artritis psoriática, artritis reumatoide, enfermedad de Crohn, esclerosis múltiple, enfermedad irritable del intestino, espondilitis anquilosante, lupus sistémico eritematoso síndrome de Sjogren. Los tipos de psoriasis incluyen psoriasis de placa, psoriasis gotosa, psoriasis pustulosa y psoriasis de las uñas. Las alergias incluyen asma y enfermedad pulmonar obstructiva crónica. La inducción de IL-10 también es útil en otras dolencias de piel crónicas, recurrentes y otras en donde esté involucrado el antígeno de linfocito cutáneo o el antígeno de leucocito cutáneo.

La composición de la proteína Arabinogalactán purificada (AGP) exhibe excelente actividad anti-psoriática *in vitro* e *in vivo*, mediada por vía de la inducción IL-10.

El AGP purificado de la presente invención (que es soluble en agua) obtenida después de cromatografía de exclusión de tamaño sobre, por ejemplo, Sefacril se constituye de varias fracciones de diferentes pesos moleculares. Tales fracciones tienen pesos moleculares promedio tan altos como entre 115 a 150 KD y tan

bajos como entre 10 a 15 KD. Los AGP que poseen altos pesos moleculares se denominan AGP-HM, mientras que aquellos de bajos pesos moleculares se denominan AGP-LM. Una composición AGP típica se constituye así de los AGP-HM y AGP-LM en proporciones variantes. Se debe entender que el AGP anteriormente mencionado se forma a través de una senda biogenética, en donde los carbohidratos/monosacáridos están constantemente buscando una proteína para formación de enlace covalente, conduciendo finalmente a los AGP de peso molecular bajo (AGP-LM) y peso molecular alto los (AGP-HM). Tal formación de enlace covalente es constantemente propagada y si un método particular termina con un AGP-LM de peso molecular promedio de menos de 10 KD o un AGP-HM de peso molecular promedio mayor de 150 KD dependería de varios factores, que son para nombrar un poco la naturaleza de las partes de la planta utilizada es decir si las hojas o los tallos frescos de la planta *Argemone mexicana* se han utilizado para extracción, las condiciones de cosecha de las hojas es decir si se cosechan en la estación de invierno etc. De esta manera, es posible que una composición AGP purificada que tiene un peso molecular promedio por fuera del rango de 10 KD 150 KD se podría obtener empleando el método de la presente invención dependiendo de los factores anteriormente mencionados. En vista de lo anterior, la composición, AGP con un peso molecular promedio por fuera del rango de 10 KD a 150 KD estaría dentro del alcance de la invención. Ejemplos de tales composiciones AGP son aquellas

con un límite inferior de un peso molecular promedio de 9 KD o composiciones AGP con un límite superior del peso molecular promedio a 150 KD.

La masa del AGP obtenida por medio del presente método está constituida de carbohidratos, con una proporción más pequeña de proteínas. Los componentes de carbohidrato del AGP son Arabinosa, Ramnosa, ácido Urónico Metilado, Manosa, Galactosa, Glucosa, y otros azúcares no identificados, que están ligados a un componente de proteína que consiste de varios aminoácidos, que son identificados, como Asparagina, Glutamina, Hidroxiprolina, Serina, Glicina, Histidina, Arginina, Treonina, Alanina, Prolina, Tirosina, Valina, Metionina, Isoleucina, Leucina, Lisina, Fenilalanina, etc., como sería evidente de las Tablas I y II de la especificación.

Se nota que no solamente es la composición glicosil de ambos AGP-HM y AGP-LM similares es decir ellos muestran similitud en la proporción de monosacáridos y proteínas presentes en esta sino el enlace de glicosilo en ambos casos son también similares.

Además, se nota que la actividad biológica de los dos componentes de peso molecular son comparables y no muestran mucha variación. Es decir, el AGP-ML purificado que tiene un peso molecular de 10 KD exhibe una o más de inmunosupresión; inhibición de

linfoproliferación; modulación de citoquina tal como inhibición de IL-2, inhibición de IFN-λ, e inducción de IL-10; inhibición de proliferación de queratinocito, actividad queratolítica, inhibición MEST y más importante actividad antisoriasis que es comparable con aquellas exhibidas por un AGP-HM purificado que tiene un peso molecular de 150 KD.

La composición AGP-de esta invención se compone de los AGP-(tanto AGP-HM como AGP--LM) que es sustancialmente libre de otros componentes presentes en el extracto de la planta, que son una mezcla de alcaloides, flavonoides, ácidos orgánicos, aminoácidos, azúcares, polisacáridos, proteínas, proteoglicanos (excluyendo AGP) y sales. Como se utiliza aquí el término "sustancialmente libre" pretende cubrir composiciones AGP que contienen desde aproximadamente 0.0001% en peso a aproximadamente 10% en peso de los otros componentes del extracto de la planta.

La fracción de n-Butanol contiene una mezcla de alcaloides (tal como protopina, nitrato deprotopina, berberina, nitrato de berberina, criptopina, alocriptopina, coptisina, sanguinarina, dihidrosanguinarina, norsanguinarina, 6-acetonil dihidrosanguinarina, dihidroqueleritrina queleritrina, norqueleritrina, norqueleritrina, 6-acetonil dihidroqueleritrina, (-) queilantifolina, (-) β-esculerina metohidróxido, (-) α-

estilopina metohidróxido, 6-acetonil dihidrosanguinarina, (-) - αteytrahidropalmatina, metohidróxido, reticulita, talifolina, muramina, argemonina, norargeminina, heleritrina, y oxihidrastinina), flavonoides (tales como isormanetina, isoramanetin-3-glucósido e isorhamnetin-3,7-diglucósido) y otros compuestos de peso molecular bajo;

La fracción soluble en metanol contiene aminoácidos (tales como histidina, lisina, ácido glutámico, glicina, alanita, leucina, valina, fenilalanina, tirosina, treonina, arginina, serina, aspargina, cisteína, metionina, triptofano, hidroxiprolina, prolina y ácido aspártico), azúcares y algunas sales;

La fracción insoluble de metanol contiene algunos ácidos orgánicos (tales como succínico, cítrico, tartárico y málico), monosacáridos, polisacáridos, proteoglicanos (incluyendo AGP) y sales.

En una modalidad de la invención, las composiciones AGP contienen menos de 1% en peso de otros componentes del extracto de la planta.

La composición AGP purificada aislada por el proceso de esta invención se encuentra que es un excelente inductor para IL-10 en

los PBMC humano activado con ConA. Este producto aproximadamente 371% de la inducción a una concentración de 200 µg /ml, que es bastamente superior a un valor de aproximadamente 171% exhibido por la fracción insoluble en metanol obtenida a través del método descrito en la publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 a la misma concentración de 200 µg /ml.

Más particularmente, la composición AGP aislada es notorio por que esta exhibe la inducción de IL-10 igual o mayor que aquella exhibida por la fracción insoluble en metanol obtenida a través del método descrito en la publicación de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 a una concentración muy baja de 0.2 a 2.0 µg/ml.

Una comparación del efecto de la concentración del AGP de la presente invención sobre la inducción del IL-10 mediante los PBMC Humanos intuidos con ConA con aquel de la fracción insoluble de metanol preparada por el método descrito en la Solicitud de Patente Estadounidense No. 2003/0194456 A1 se resume en la Tabla IA.

TABLA IA

Comparación del Efecto de la Composición AGP de la Presente Invención Sobre la Inducción de IL-10 por medio de los PBMC Humanos Inducidos con ConA con aquel de la Fracción Insoluble en Metanol Preparada Mediante el Método Descrito en la Solicitud de Patente Estadounidense No. 2003/0194456 A1.

Sr. No.	*Concentración de la Composición AGP de la Presente Invención (µg/ml)*	*Inducción Porcentual Promedio*	*Concentración de la Fracción Insoluble en Metanol obtenida mediante el Método de la Solicitud U.S. No. 2003/0194456 A1*	*Inducción Porcentual Promedio (Dada en la tabla 8 de la Solicitud US de la No. 2003/0194456 A1)*
01	200.00	371.40	200.00	171.10
02	20.00	303.80	20.00	162.10
03	2.00	237.30	2.00	98.30
04	0.20	137.20	0.20	24.60
05	0.02	114.30	0.02	-4.00
06	0.002	106.00	0.002	-5.00
07	0.0002	102.60	0.0002	--

* Los valores son descritos en incremento porcentual del basal con referencia al control.

** El porcentaje promedio de inducción exhibido por la fracción insoluble en metanol obtenida a través del método descrito en la de la Solicitud de Patente Estadounidense No. 2003/0194456 A1 fue de 171% a una concentración de 200 µg/ml.

Tal actividad bastamente potente exhibida por el AGP obtenido por el método descrito anteriormente le posibilita ser administrada a una concentración sustancialmente reducida de 1/centésima a 1/milésima de la dosis requerida a ser administrada utilizando los extractos/fracciones obtenidas por el método descrito en la Solicitud de Patente Estadounidense No. 2003/0194456 A1 suministrando de esta menara un tratamiento efectivo en costos, eficiente y bien tolerado para la psoriasis y otros trastornos.

La presente invención suministra composiciones farmacéuticas que comprenden una cantidad efectiva de la composición de proteína Arabinogalactán (AGP) purificada, que tiene un rango de peso molecular promedio entre 10 KD a 150 KD, aislada de las hojas y/o los tallos de la planta de *Argemone mexicana* mediante el método selectivo enumerado anteriormente, en mezcla con excipientes farmacéuticamente aceptable. Las composiciones de esta invención son seguras, efectivas y bien toleradas.

Las composiciones farmacéuticas adecuadas para uso en la presente invención incluyen composiciones en donde los ingredientes activos están contenidos en una cantidad efectiva para lograr su propósito deseado. El término una cantidad efectiva significa que la cantidad de la composición AGP que elicitará la respuesta biológicas o médica de un tejido, célula, o sistema, animal, mamífero no humano o mamífero humano que está siendo buscada. Se

pretende referirse a situaciones donde abría desaceleración, interrupción, tensión o parada de la progresión de las enfermedades y/o condiciones descritas aquí, pero no necesariamente indican una eliminación total de toda la enfermedad y si tomas de de la condición, sino que incluyen tratamiento profiláctico de enfermedades y/o condiciones.

La composición AGP de la presente invención se puede formular en una forma de dosis adecuada en mezcla con uno o más excipientes farmacéuticamente aceptables tales como vehículos, diluyentes llenadores y similares. Las formas de dosis adecuadas son formas adecuadas para la administración oral o la aplicación tópica. Ejemplos no limitantes en tales formas de dosis son líquidas, polvos secos o concentrados de polvo, cápsula, tableta, glóbulos, gránulos, geles, ungüentos, cremas, emulsiones, suspensiones, dispersiones, lociones, píldoras y similares.

Generalmente una composición farmacéutica típica para la administración oral comprende la composición AGP como el ingrediente activo en una cantidad en el rango de entre 50-5.000 mg, más preferiblemente 200 mg.

Generalmente una composición farmacéutica típica para la administración tópica comprende la composición AGP como el

ingrediente activo en una cantidad en el rango de entre 0.1-10% en peso, más preferiblemente 2% en peso.

La dosis profiláctica o terapéutica de la composición AGP o las composiciones que contienen la composición AGP es de 50 mg a 5000 mg por día preferiblemente 200 mg de dosis por día. La dosis se puede administrar en una dosis simple o dividida.

Sin embargo, la formulación exacta como la ruta de administración y la dosis se pueden escoger por el paciente o el profesional al cuidad de la salud en vista de la condición del paciente y si el paciente está actualmente afligido de la enfermedad o condición o si el tratamiento es profiláctico. Una dosis profiláctica se puede administrar a un paciente que este en riesgo de desarrollar una enfermedad o condición. Los factores riego incluyen pero no están limitados a factores genéticos y de riesgo ambiental. Se refiere administrar la composición farmacéutica a una dosis que producirá el resultado deseado son originar efectos colaterales indebidos, como se utilizan aquí, el término "paciente" significa animal, mamífero no humano, o mamífero humano.

Se pueden preparar formas de dosis adecuadas para la administración oral y la aplicación tópica que comprendan la composición AGP de la presente invención en mezcla con vehículos farmacéuticamente aceptables.

Las formas adecuadas de administración oral incluyen tabletas, cápsulas, concentrados en polvo, melazas, elixires o suspensiones. Las formas adecuadas de aplicación típica incluyen ungüentos, cremas lociones, aceites y sistemas de suministro de droga transdérmica.

Los excipientes farmacéuticamente aceptables adecuados incluyen azúcares tales como lactosa, sucrosa, manitol, sorbitol, y xilitol; Almidones tales como almidón de maíz, almidón de tapioca y almidón de papa; Celulosa y sus derivados tales como carboximetil celulosa de sodio, etilcelulosa y metilcelulosa; Fosfatos de calcio tales como fosfato de dicalcio y fosfato de tricalcio; sulfato de sodio; Sulfato de calcio; Polivinil pirrolidona; alcohol polivinílico; Ácido esteárico; aceites vegetales tales como aceite de maní, aceite de semilla de algodón, aceite de sésamo, aceite de oliva y aceite de maíz; Tensoactivos no iónicos, catiónicos y aniónicos; Polímeros de etilenglicol; β-ciclodextrina, Alcoholes grasos; Sólidos de cereal hidrolizados; Así como también otros llenadores compatibles no tóxicos, Ligadores, Desintegrantes, Amortiguadores, Preservativos, Antioxidantes, Lubricantes, agentes Saborizantes etc.

Las composiciones de la invención son preobradas de acuerdo a técnicas convencionales conocidas en el arte.

Las composiciones son farmacéuticamente aceptables significando que ellas son adecuadas para el uso con humanos y/o animales.

Las composiciones AGP y las composiciones farmacéuticas de esta invención son útiles en el tratamiento y/o la profilaxis de las enfermedades y condiciones descritas aquí.

La composición AGP de esta invención o una composición farmacéutica que comprenda la composición AGP de esta invención se puede utilizar en el tratamiento y/o la profilaxis de dolencias de psoriasis de la piel tales como psoriasis incluyendo psoriasis de placa, psoriasis gotosa, psoriasis pustulosa y psoriasis de las uñas que comprende la administración a un mamífero necesitado de tal tratamiento o a un mamífero en riesgo de dolencias de psoriasis en la piel de una cantidad efectiva de la composición AGP o una composición farmacéutica que comprenda una cantidad terapéuticamente efectiva de la composición de proteína Arabinogalactán purificada (AGP).

La composición AGP o la composición farmacéutica que comprende la composición AGP se puede utilizar en el tratamiento o profilaxis de trastornos inflamatorios, enfermedades autoinmunes como

artritis psoriática, artritis reumatoide, enfermedad de Crohn, esclerosis múltiple, enfermedad del intestino irritable, espondilitis anquilosante, lupus sistémico eritematoso y síndrome de Sjogren; alergias como el asma y la enfermedad pulmonar obstructiva crónica. El tratamiento o la profilaxis comprenden la administración a un mamífero necesitado de tal tratamiento o a un mamífero en riesgo de desarrollar o experimentar un ataque de uno de estos trastornos o enfermedades una cantidad efectiva de la composición AGP o una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la composición de proteína Arabinogalactán purificada (AGP).

Las composiciones de esta invención que originan la inmunosupresión, también son útiles para pacientes que esperan sufrir o han recibido un trasplante de órgano.

Elucidación Estructural y Caracterización de la Composición AGP.

Determinación del Contenido de Carbohidratos de la Composición AGP.

El contenido de carbohidrato total de las composiciones AGP, de la presente invención se determinó mediante el método de ácido fenol-sulfúrico utilizando D-galactosa como un estándar. La composición AGP, AGP-HM y AGP-LM se disolvieron separadamente en

una concentración de 0.16 mg/ml con agua destilada. A 1 ml de cada una de estas tres soluciones tomadas separadamente se agregó 1 ml de solución de fenol a, 5% Y 10 ml de ácido sulfúrico. Estos fueron sometidos a vértice para mezclarlos permitiéndose el enfriamiento a temperatura ambiente. La absorbancia se midió por 280 nm. El contenido de carbohidrato de las composiciones AGP se encuentra que está entre 45 -98% respectivamente comparado con el estándar de galactosa.

Determinación del Contenido de Proteína de la Composición AGP.

El contenido de proteína total de las composiciones AGP, de la presente invención se determina mediante el método Bradford. El contenido de la proteína de la compresión AGP, AGP-HM y AGP-LM estuvo entre 2-20%. Los constituyentes de aminoácido de la composición AGP se identifican como asparagina, glutamina, hiodroxiprolina, serina, glicina, histidina, arginina, treonina, alanita, prolina, tirosina, valina, metionina, isoleucina, leucina, lisina, y fenilalanina, por medio del analizador de aminoácido utilizando estándares.

La identificación de Glicosilo y el contenido de Aminoácido de la composición AGP por Hidrólisis y TLC 20 mg de fracción neutra obtenida después de cromatografía de exclusión de tamaño se tomó y se Hidrolizó con 2M de ácido trifluoro acético (TFA, 200 µl) en

un frasco cerrado. El frasco se calentó a 100°C durante 1 h, se concentró y se liofilizó. Una Cromatografía de Capa Delgada (TLC) de la mezcla se corrió con azúcar y estándar y las muestras de aminoácido. Los resultados observados mostraron la presencia de monosacáridos tales como arabinosa, galactosa, glucosa, ramnosa, y manosa. Los aminoácidos tales como valina, fenilalanina, serina, GABA, isoleucina e histidina también se observaron.

Identificación del Contenido de Glicosilo y de la Composición AGP

El análisis d la composición de glicosilo se desarrolló mediante cromatografía de gas/espectrometría de masa combinadas (GC/MS) de los derivados de per-*O*-trimetilsilil (TMS) de los metil glicósidos de monosacáridos producidos del AGP-HM y del AGP-LM mediante metanolisis ácida.

Los metil glicósidos fueron primero preparados de la muestra seca de los AGP mediante metanólisis en HCL en 1 M en metanol a 80°C (18-22 horas) seguido por la re-*N*-acetilación con piridina y anhídrido acético en metanol (para la detección de azúcares amino). Las muestras fueron entonces per -*O*-trimetilsiladas por el tratamiento con Tri-Sil (Pierce) a 80°C (0.5 horas). Estos procedimientos se llevaron a cabo tal como se describió previamente por Cork, W. S., Darvill, A. G., McNeil, M.,

Stevenson, T. T., y Albersheim, P., *Methods Enzymol.*, 1985, 230, 1-15 y *Methods Enzymol.*, 1985, 118, 3-40.

Los análisis GC/MS de los metil glicósidos TMS se desarrollan sobre un HP 5890 GC en interfase con un 5970 MSD, utilizando una columna capilar de sílice fusionada a EC-1 All Tech (30mm x 0.25 mm ID).

La Tabla I resume los componentes de glicosilo de la composición AGP.

Tabla I

Componentes de glicosilo de la composición AGP

Componentes	***Composición AGP (Mol %)***
Arabinosa (Ara)	34.00
Ramnosa (Rha)	3.40
Ácido urónico metilado (MUA)	5.00
Manosa (Man)	2.20
Galactosa (Gal)	48.90
Glucosa (Glc)	No detectado
Azúcares no identificados	6.50

Determinación del Enlace de Glicosilo de la Composición AGP

Método NaOH: Para el análisis de enlace de glicosilo, una muestra de la composición AGP se permetiló, despolimerizó, se redujo, y acetiló; y los alditol acetatos parcialmente metilados resultantes (PMAA) analizados mediante la cromatografía-espectrometría de masa (GC-MS) como se describió antes [Cork, W. S., Darvill, A. G., McNeil, M. Stevenson, T. T. y Albersheim, P., **Methods Enzymol.**, 1985, 230, 1-15; Cork, W. S. Darvill, A. G., McNeil, M., Stevenson, t. T. and Albersheim, P., *Methods Enzymol.*, 1985, 118, 3-40].

Inicialmente, una alicota de la muestra se permetiló [mediante el método de Ciukanu y Kerek, *Carbohydr. Res.* 1984, 131, 209-217] incluyendo el tratamiento con hidróxido de sodio y yoduro de metilo en DMSO. La permetilación se repitió 2 veces con el fin de ayudar a completar la mutilación del polímero. Luego del trabajo de la muestra, el material permetilado se hidrolizó utilizando ácido trifluoroacético 2 M (2 h en tubo sellado a 121°C), producido por $NaBD_4$, y y acetilado utilizando anhídrido acético/piridina. Los PMAA resultantes fueron analizados en un Hewlett Packard 5890 GC e interfase con un 5970 MSD (detector selectivo de masa; modo de ionización de impacto electrón); la separación se desarrolló sobre una columna capilar de sílica fusionada de fase unida a 30 m Supelco 2330.

La Tabla II resume los enlaces glicosilo con los porcentajes.

Tabla II

Enlace Glicosilo de la Composición AGP

Residuo Glicosilo	***Porcentaje Presente en la Composición AGP***
Residuo de ramnopirasonil Terminal (t-Rha *p*)	3.00
Residuo de arabinofuranosilo Terminal (t-Ara *f*)	22.00
Residuo de arabinopiranosilo Terminal (t-Ara *p*)	1.00
Mezcla Residuo de 3-ramnopiranosilo (3-Rha *p*) Residuo de 4-ramnopiranosilo (4-Rha *p*)	1.00
Residuo de glucopiranosilo Terminal (t-Glc *p*)	3.00
Residuo de galactopiranosilo Terminal (t-Gal *p*)	5.00
Residuo de arabinofuranosilo 5-ligado (5-Ara *f*)	5.00
Residuo de manopiranosilo 2-ligado (2-Man *p*)	Trace
Residuo de glucopiranosilo 2-ligado (2-Glc *p*)	Trace
Residuo galactopiranosilo 3-ligado (3-Gal *p*)	16.00

Residuo galactopiranosilo 4-ligado (4-Gal *p*)1.00	1.00
Residuo glucopiranosilo 6 - ligado (6-Glc *p*)	2.00
Residuo galactopiranosilo 6-ligado (6-Gal *p*)	8.00
Residuo glucopiranosilo 3,6-ligado (3,6 -Glc *p*)	2.00
Residuo galactopiranosilo 3,6-ligado (3,6-Gal *p*)	31.00

Determinación del Peso Molecular de la Composición AGP

La composición AGP, AGP-HM y AGP-LM se analizaron utilizando el instrumento GPC acuoso Waters, Modelo Alliance 2690 que está equipado con un solvente integrado, la unidad de manejo demuestra y el detector de índice refractivo que contiene celdas & ópticos de flujo térmicamente protegido con un intercambiador de calor de contracorriente para una mejor estabilidad de la línea base. La estación de trabajo de cromatografía incluye la adquisición de datos y el software de control y el software Milenium para el procesamiento de datos. Las columnas GPC son de Tosoh Corporation (tipo TSK-GEL PW) que consiste del gel semi rígido basado en polímero hidrofílico. E límite de exclusión de los polisacáridos (Dextrano) es de 1,000 a 7,000,00 de peso molecular. Estas columnas fueron designadas para separación analítica y

preparativa de los polímeros sintetizados solubles en agua, los oligómeros y las sustancias biológicas tales como los polisacáridos, los ácidos nucleicos, las proteínas, los péptidos, etc. El kit pullulan consiste de muestras de polisacárido de diferente peso molecular para la calibración de columna.

El método GPC se ha desarrollado utilizando condiciones analíticas variantes tales como columnas de porosidad diferentes, concentración & naturaleza de la fase móvil, taza de flujo, sensibilidad del detector etc. La separación activa con la muestra de polisacárido se obtiene al utilizar solución de nitrato de sodio 0.2 M y las siguientes fueron las condiciones analíticas adecuadas para la caracterización de la molécula de droga basada en polisacárido.

El peso molecular de la composición AGP se determinó por comparación del tiempo de elusión utilizando cromatografía de exclusión de tamaño. Con base en la comparación del rango de peso molecular promedio de la composición AGP se encuentra que está entre 10 KD a 150 KD.

Los pesos moleculares del AGP-LM y AGP-HM también se determinaron de una manera similar. Con base en la comparación del peso molecular promedio de los componentes se encuentra que es de 13 KD a 15 KD respectivamente.

Espectro Infrarrojo de Transformación Fourier (FT-IR) DE LA composición AGP.

El espectro IR de la AGP-HM de la presente invención medido por el Espectrómetro Infrarrojo de Transformación Fourier (8201 PC Shimadzu) utilizando muselina Nujol en glóbulos KBr muestra banda de absorción amplia a 3400 cm^{-1} que indica la presencia de gripos hidroxilo y a 2850-2960 cm^{-1} revelando el tensionamiento de los enlaces CH.

H) Espectro Magnético Nuclear ^{1}H de la Composición AGP

El espectro NMR ^{1}H delAGP de la presente invención se registra en D_2O a 500 MHz en un Espectrómetro de Resonancia Magnética Nuclear broker DRX 500.

Se hizo una asignación de protón extensiva de todos los principales tipos de enlace detectados mediante el análisis de mutilación. En el espectro RMN ^{1}H las señales H-1 corresponden a los residuos de β-D-Gal-*p* (δ 4.22-4.47) y residuos de α-L-Ara-*f* (δ 5.17-5.33) se detectaron. Hubo numerosas señales de δ 3.53-4.22, que corresponden a H-2 a H-6 de los residuos β-D-Gal-*p*, H-2 a H-5 de los residuos α-L-Ara-*f* y H-2 A H-5 de los residuos pequeños de glucosa, ramnosa y manosa. Para resolver y asignar

estas señales se desarrollan experimentos 2D homo- y heteronucleares para residuos particulares. La asignación de los diferentes tipos de enlace para cada uno de los sistemas spín identificado en el espectro 2D se soporta por los datos de enlace en la tabla II y por comparación con los datos RMN reportados en la literatura pata otros AGP.

Protones anoméricos: Los protones anoméricos observados en δ 5.33 y δ 5.17 se asignan al terminal y los residuos α-L-Ara*f* 5-enlazados, respectivamente. La señal en δ 5.17 también se asigna al terminal α-L-Rha *p* y δ 5.10 al α-L-Rha *p* 4-ligado. De forma similar, la señal en δ 4.61 se asigna a α-L-Rha *p* 3-ligado. La señal en δ 4.56 se asigna a β-D-Gal *p*. Signal 6-ligado. La señal en δ 4.61 se asigna β-D-Gal *p* 3-ligado y las señales en δ 4.56 y 4.57 se asignan al terminal β-D-Gal *p* y β-D- Gal *p*.3- 6-ligado.

Protones H-2: Las señales en δ 4.31 y 4.22 se asignan a H-2 del terminal y el residuo α-L-Ara *f* 5-ligado, respectivamente. Adicionalmente, la señal en δ 3.45 se asigna al terminal β-D-Gal *p* mientras que la señal en δ 3.74 se asigna a los residuos β- D-Gal *p* 3-, 6- y 3,6- ligado. Las señales en δ 4.03, 3.74 y 3.57 se asignan a los protones H-2 de 4-ligado, 3-ligado y el terminal α-L-Rha *p*, respectivamente.

Protones H-3: Señales en δ 4.03 se asignan al terminal y los residuos α-L-Ara *f* 5-ligados. Adicionalmente la señal en δ 3.81 se asigna al terminal y el β-D-Gal *p* ligado. De forma similar la señal en δ 4.31 se asigna a las porciones β-D-Gal *p* 3- y 3,6-ligado. Las señales en δ 3.93, 3.63 y 3.57 se asignan a 4-ligado, terminal y α-L-Rha *p* 3-ligado, respectivamente.

Protones H-4: La señales en δ 4.22 se asigna al terminal y al α-L-Ara *f* 5-ligado. La señal en δ 3.63 se asigna a los residuos β-D-Gal *p* 3- y 6-ligados. Adicionalmente, las señales en δ 3.86 y 4.03 se asignan al terminal y al β-D-Gal *p* 3,6-ligado, respectivamente. De forma similar, las señales en δ 3.63, 3.09 y 2.82 se asignan a la porción α-L-Rha *p* terminal, 4-ligada, 3-ligada.

Protones H-5: La señal en δ 3.81 se asigna a β-D-Gal *p* 6- y 3, 6-ligado. De forma similar la señal en δ 4.03 se asigna al terminal y β-D-Gal *p* 3-ligado. Las señales en δ 3.91 y 3.93 se asignan al terminal y al α-L-Ara *f* 5-ligado respectivamente. Las señales en δ 4.22, 4.03 y 3.09 se asignan al H-5 del terminal, α-L- Rha *p* 4-ligado y 3-ligado. Las señales en δ 4.03, 3.45 y 4.03 se pueden asignar a H-5 de β-D-Gal *p* 3-ligado y los residuos β-D-Gal *p* terminal, respectivamente.

Protones H-6: La señal en δ 3.93 se asigna a β-D-Gal *p* 6- y 3, 6-ligado. Las señales en δ 3.91 y 3.81 se asignan al terminal y al β-D-Gal *p* 3-ligado, respectivamente. Los protones de metilo observado en δ 1.03, 1.34 y 1.40 son asignables a la porción α-L-Rha *p* terminal y 3-ligado, 4-ligado.

Las anteriores asignaciones se confirman adicionalmente al llevar a cabo experimentos HOMOCOSY.

Residuos de aminoácidos: El grupo metilo para los aminoácidos valina, leucina e isoleucina se observan en δ 1.03 mientras que los metilos asignables a treonina y alanina se ubican en δ 1.34 y 1.40, respectivamente.

Los protones Cα, Cβ (C-H, CH_2), Cγ (CH_2) y Cδ (CH_2) de arginina, asparagina, glutamina, isoleucina, leucina, lisina, metionina, prolina y valina se observan entre δ 1.3-2.8.

Los protones C-β (CH_2, CH), Cγ, Cδ de asparagina, histidina, fenilalanina y tirosina se observan entre δ 2.8-3.5.

Los protones Cα de alanina, arginina, glutamina, glicina, isoleucina, leucina, lisina, metionina, treonina y valina se observan entre δ 3.5-3.9.

Los protones Cα de asparagina, histidina, fenilalanina, prolina, serina, tirosina y protones Cβ de serina y treonina se observan entre δ 3.90-4.2.

Señales débiles en la región de campo descendente de δ 6.6-7.5 se asignan a protones N-H y aromáticos de fenilalanina y tirosina.

i) Espectro Magnético Nuclear ^{13}C de la Composición AGP

Carbonos Anoméricos: En ell espectro RMN 13C las señales en δ 109.10 y 107.37 se asignan a los carbonos anoméricos del terminal y la porción α-L-Ara *f* 5-ligada. Las señales entre δ 103 -102 se asignan a carbonos anoméricos de terminal, 3- ligada, 6-ligada, y 3, 6-ligada de residuos β-D-Gal *p*. Las mismas señales traslapantes entre δ 103-102 se asignan a carbonos anoméricos de β-D-Glu *p* 4-ligada, β-D-xyl *p* 4-ligada terminal β-D-Glc *p* y α-L-Rha *p* 3-ligada.

C-2: Las señales en δ 81.97 se asignan α-L-Ara *f* 5-ligada. Las señales para C-2 observadas en δ 72.94 y 72.72 se asignan al terminal y a los residuos β-D-Gal *p* 6-ligado, respectivamente. Las señales en δ 72.56 se asignan a residuo β-D-Gal *p* 3,6-ligado. De forma similar, las señales C-2 asignables al terminal y al β-D-Glc *p* 6-ligado se observan en δ 74.81, 74.60 y 73.33,

respectivamente. Las señales C-2 en los residuos asignables α-L-Rha *p* al terminal, 3-ligado y 4-ligado se observan en δ 80.02, 76.55 y 70.68, respectivamente. La señal en δ 81.24 se asigna al residuo β-D-Man *p* 2-ligado.

C-3: Señal en δ □ 76.55 se asigna al terminal y el α-L-Ara *f* 5-ligado. □ La señal en δ74.81 se asigna al terminal y el D-Gal *p* 6-ligado. Adicionalmente, la señal en δ 81.97 se asigna al residuo β-D-Gal *p* 3- y 3,6-ligado. Las señales en δ 76.55 y 74.81 se asignan al terminal, las porciones β-D-Glc *p* 4-ligado y 6-ligado. De forma similar, las señales en δ 80.02, 76.55 y 70.6 se asigna al terminal, la porción α-L-Rha *p* 3-ligado y 4-ligado, respectivamente.

C-4: La señal en δ 83.85 se asigna al terminal y a porción α-L-Ara *f* 5-ligado. La señal en δ 70.15 se asigna al terminal residuo β-D-Gal *p*. La señal en δ 70.68 se asigna al residuo β-D-Gal *p* 3- y 6-ligado. Mientras que la señal en δ 73.33 se asigna al residuo β-D-Gal *p* 3,6-ligado. Las señales en δ 80.02, 70.68 y 70.15 se asignan a C-4 de la porción β-D-Glc *p* 6-ligada y terminal, respectivamente. Las señales en δ 81-83 se asignan a C-4 de la porción 3-ligada, 4-ligada y terminal.

C-5: Señal debido a C-5 de α-L-Ara *f* 5-ligada se observa en δ 69.17 mientras que del α-L-Ara *f* terminal se observa en δ 61.25. Las señales C-5 de debidas al residuo β-D-Gal *p* terminal, 3-ligado, 6-ligado se observan entre δ 74-77, mientras, las señales C-5 debidas al terminal se observan en δ 75-76 en el caso de la porción β-D-Glc *p*.

C-6: Las señales C-6 debidas a los residuos β-D-Gal *p* y 3, 6-ligados se observan en δ 69.17 mientras que las señales 3-ligadas y terminal se observan en δ 60.93 y 61.25, respectivamente. La señal debida al C-6 en los residuos β-D-Glc *p* para el terminal se observan entre δ 60-62 y la señal en δ 70.1 se observa para 6-ligado.

Metilos: Las señales observadas en δ 16.7, 19.9 y 22.2 se asignan a los grupos metilo de α-L-Rha *p*.

Las anteriores asignaciones se confirman adicionalmente al llevar a cabo experimentos HETCOR.

Aminoácidos: La señal en δ 16.70 se asigna a metilos C γ y Cδ de valina, metilo Cβ de alanina y metilo Cδ □ de metionina. La señal en δ 19.97 se asigna a metilo Cγ de treonina. La señal en δ 22.22

se asigna a Cγ (CH_2), Cγ (C-H) de arginina, leucina, isoleucina, lisina y a metilos Cδ y Cε de leucina.

La señal en δ 31.01 se asigna a Cβ (CH_2) de arginina, metionina, lisina, prolina y Cγ (CH_2) de glutamina.

La señal en δ 38.05 se asigna a Cβ (CH_2) de asparagina, leucina, fenilalanina, tirosina, Cα de glicina y Cδ (CH_2) de arginina y lisina. La señal en δ 48.45 se asigna al protón Cα de alanina, asparagina, leucina y Cδ de prolina.

La señal en δ 59.81 se asigna a protones Cα de arginina, cistina, glutamina, histidina, isoleucina, lisina, metionina, fenilalanina, serina y tirosina. La señal en δ 60.9 se asigna al protón Cα de valina y treonina mientras que las señales 61.25 se asignan al protón Cα de prolina y al protón Cβ de serina. La señal en δ 68.38 se asigna a Cβ (CH) de treonina.

Las señales entre δ 108-140 se asignan a los protones olefínicos de histidina y protones aromáticos de fenilalanina.

La señal en δ 155.64 se asigna a Cε de arginina y C-4 de tirosina mientras que la señal de carbonilo en δ 166.19 se asigna a glutamina.

En la región de campo descendente las señales observadas entre δ 171 - 175 se asignan a los grupos carbonilo de los aminoácidos alanina, arginina, asparagina, glutamina, glicina, histidina, isoleucina, leucina, lisina, metionina, fenilalanina, prolina, serina, treonina y tirosina.

En conclusión la estructura del AGP-HM consiste de galactopiranosa 6-ligado y galactopiranosa 3, 6-ligado como la estructura y arabinofuranosilo, arabinopiranosilo, ramnopiranosilo, glucopiranosilo y residuos galactopiranosilo en las posiciones terminales. El AGP adicional contiene □ramnopiranosilo 3-ligado, 4-ligado, mannopiranosilo 2-ligado, glucopiranosilo 2-ligado, galactopiranosilo 3-ligado, 4-ligado, glucopiranosilo 6-ligado, 3,6-ligado y residuos xilopiranosilo 4-ligados junto con ácido metil urónico. Los constituyentes de aminoácido de la composición AGP se identifican como asparagina, glutamina, hidroxiprolina, serina, glicina, histidina, arginina, treonina, alanina, prolina, tirosina, valina, metionina, isoleucina, leucina, lisina y fenilalanina. Sin embargo, los sitios de adhesión de los aminoácidos no se han determinado. El AGP-LM también posee similar estructura pero difiere en el peso molecular. Basado en los datos mencionados anteriormente se ha propuesto la siguiente estructura para la composición AGP (Fig. 2). El AGP-HM y el AGP-LM poseen similar actividad biológica, por

lo tanto, la composición AGP se ha empleado para todos los estudios biológicos.

Las propiedades inmunológicas y farmacológicas de la composición AGP

El ensayo de citoquina, tal como IL-2, IFN-γ, IL-IO y otras actividades in vivo tal como hipersensibilidad del tipo retrasado (DTH) en conejillos de indias se describe adelante. Una comparación de actividades biológicas de extracto acuoso como se obtienen mediante el método descrito en la Publicación de solicitud Estadounidense No. 2003/0194456 A1 y la composición AGP de la presente invención se dan en la Tabla-III.

Tabla III

Eficacia biológica comparativa de extracto (1) acuoso obtenido mediante el método descrito en la Publicación de solicitud Estadounidense No. 2003/0194456 A1 ***y en la composición AGP de la presente invención (2)***

Serie No.	***Parámetros***	***Extracto Acuoso (1)***	***Composición AGP (2)***
1	Inducción IL-10 (EC_{50} en µg/ml)	2.54	1.075
2	Inhibición IL-2 (%	29.32±5.26	51.10±4.33

	de inhibición en 20ng/ml)		
3	Inhibición IFNγ (% de inhibición en 20ng/ml)	33.27±6.52	54.50±4.00
4	Inhibición TNFα (% de inbición en 2µg/ml)	44.34±6.64	60.68±9.03
5	Inhibición GMCSF (% de inhibición en 200 ng/ml)	35.57±9.45	43.28±6.21
6	Hiperplasia de piel inducida por TPA (ED_{50} mg/kg)	36.3	2.31
7	ModeloDTH (ED_{50} mg/kg)	4.34	0.58
8	MEST (ED_{50} mg/kg)	14.0	6.43

Estudios Toxicológicos en la composición AGP

La toxicidad aguda (LD_{50}) de la composición AGP se evalúa en ratones y ratas por ruta oral e i.v. Grupos de diez (10) animales de cada especie por ruta por dosis se medica y los resultados se calculan en el día 15.

Los siguientes valores se observan para la composición AGP.

LD_0 de ratón p.o. : >5000mg/kg b.wt.

LD_0 de ratón i.v. : >1000mg/kg b.wt.

LD_0 de rata p.o. : >5000mg/kg b.wt.

LD_{50} de rata i.v. : >250mg/kg b.wt.

Ensayo biológico de la composición AGP

Descripción de los Métodos de Evaluación para Producción de Human IL-10

Con el fin de evaluar la eficacia de la composición AGP para su potencial terapéutico en psoriasis, su papel en la inducción IL-10 en Vitro se avalúa por el ensayo de producción IL-10 con PBMC inducido por ConA [Raychudhuri S. P., Farber E. M., Raychudhuri S. K., *Int. J. Immunopharmacol.*, 1999, 21,609].

En resumen, el PBMC humano (Células homonucleares sanguíneas periféricas) se separan y se estimulan con 10 µg/ml ConA junto con varias concentraciones de la composición AGP y se incuban durante 48 horas a 37° C en un incubador de CO_2 con 5% CO_2. Se cosechan los supernadantes y se congelan a -70° C hasta la ecuación ELISA. Se utilizan kits ELISA IL-10 humano de R and D System para la detección del IL-10 en supernadante de cultivo. El porcentaje de inducción se calcula con referencia al control. Los resultados se resumen en la Tabla-IV.

La composición AGP aislada del *Argemone mexicana* exhibe incremento en la producción del IL-10 en PBMC humano activado por ConA en el rango de 0.0002 µg/ml a 200 µg/ml (Fig. 3). Se

encuentra que el IL-10 es regulador de citoquina en el tratamiento de la psoriasis y también se conoce por inducir terapia anti-psoriática.

La inducción IL-10 es útil en el tratamiento y/o profilaxis de psoriasis, dermatitis, escleroderma, trastornos inflamatorios y otras enfermedades autoinmunes como artritis psoriática, psoriasis de placa , psoriasis gotosa, artritis reumatoide, enfermedad de Crohn, esclerosis múltiple, enfermedad del intestino irritable, espondilitis anquilosante, lupus eritematoso sistémico, síndrome de Sjogren, alergias similares a asma, enfermedad pulmonar obstructiva crónica y condiciones relacionadas tal como eczema y dermatitis atópica. La inducción del IL-10 también es útil en otras insuficiencias crónicas de la piel, recurrentes, en donde se involucra el antígeno de linfocito cutáneo o el antígeno de leucocito cutáneo.

Tabla IV

Efecto de la composición AGP en inducción IL-10 por PBMC humano inducido por ConA.

Concentración de Extracto (µg/ml)	*Promedio % de Inducción*
200	371.40
20	303.80
2	237.30
0.2	137.20
0.02	114.30
0.002	106.00
0.0002	102.60

*Los valores se describen en incremento de porcentaje de basal con referencia a control.

Descripción de los Métodos de Evaluación para Producción IFN-γ y IL-2 humano.

Con el fin de la eficacia de la composición AGP para su potencial terapéutico en psoriasis, se evalúa su papel in vitro de la modulación IL-2 y IFN-γ mediante el ensayo de inhibición de producción IFN-γ e IL-2 con PBMC inducido (PHA) fitoemaglutina [Brynskov J, Tvede N., *Gut*, 1990, 31(7), 795].

El objetivo de éste estudio es evaluar el efecto del AGP sobre la producción IL-2 e IFN-γ inducida por PHA de linfocito humano. En resumen, se obtiene celulas mononucleares sanguíneas periféricas (PBMC) de individuos saludables. Un millón de PBMC por ml se estimula con PHA (5 µg/ml) junto con varias concentraciones de composición AGP aislada de *Argemone mexicana*

durante 48 horas a 37° C en incubador de CO_2 con 5% de CO_2. Los supernadantes se cosechan y se congelan a-70° C. Se utilizan kits ELISA IFN-γ Humano e IL-2 Humano de R and D System para detección de IL-2 e IFN-γ en supernadante de cultivo. Se calcula el porcentaje de inhibición con referencia al control. La composición AGP aislada de hojas y/o tallos de *Argemone mexicana* se encuentra inhibidor para la producción IL-2 inducida por mitógeno entre 0.002µg/ml a 20µg/ml (Fig. 4). Ésta actividad inhibidora para la producción IL-2 de mitógeno se conoce por ser inmunosupresora y bien establecida por ser útil en el tratamiento y/o profilaxis de psoriasis. Los resultados se resumen en la Tabla V.

Tabla V

Efecto de la composición AGP en la producción IL-2 mediante PBMC humano inducido por PHA

Concentración de Composición AGP (µg/ml)	*Promedio % Inhibición*
20.00	54.53
2.00	54.49
0.20	53.97
0.02	51.10
0.002	30.56
0.0002	10.87

0.00002	3.75

*Los valores se describen en porcentaje de inhibición con referencia al control.

La composición AGP aislada de *Argemone mexicana* se encuentra inhibidora para la producción IFNγ inducida por mitógeno en el tango de 0.0002 µg/ml a 2µg/ml (Fig. 5). Ésta actividad inhibidora para la producción IFN-γ inducida por mitógeno se conoce por ser inmunosupresora y es bien establecida por ser útil en el tratamiento y/o profilaxis de psoriasis. Los resultados se resumen en la Tabla VI.

Tabla VI

Efecto de las Composiciones AGP en la Producción IFN-γ por PBMC Humano inducido por PHA

Concentración de Composición AGP (µg/ml)	***Promedio % Inhibición***
20.00	55.19
2.00	57.07
0.20	53.69
0.02	54.40
0.002	38.62
0.0002	15.34
0.00002	4.04

*Los valores se describen en porcentaje de inhibición con referencia al control

Descripción de los Métodos de Evaluación para la producción de TNF-alfa y GMCSF Humano

Con el fin de evaluar la eficacia de las composiciones AGP de ésta invención para su potencial terapéutico en psoriasis, se evalúa su papel en la modulación in vitro de el factor de estimulación de colonia de macrófago de granulocito (GMCSF) y TNF-α mediante el ensayo de inhibición de producción de GMCSF y TNFγ con PBMC estimulado por ConA y LPS.

En resumen, el PBMC Humano se separa de voluntarios saludables, estimulados con 10 µg/ml de ConA junto con varias concentraciones de composición AGP e incubado durante 48 horas a 37° C en incubador de CO_2 con 5% CO_2. 5 µg/ml de LPS se agrega e incuba durante 24 horas bajo las mismas condiciones. Los supernadantes se cosechan y congelan hasta cuantificación de citoquinas utilizando ELISA. El porcentaje de inhibición se calcula con referencia al control.

La composición AGP aislada del *Argemone mexicana* se encuentra inhibidor para la producción GMCSF inducida por mitógeno en el rango de 0.02 µg/ml a 2µg/ml (Fig. 6). Ésta actividad inhibidora para la producción de GMCSF inducida por mitógeno se conoce por ser inmunosupresora y bien establecida por ser útil en el

tratamiento y/o profilaxis de psoriasis. Los resultados se resumen en la Tabla- VII.

Tabla VII

Efecto de la Composición AGP en la producción de GMCSF mediante PBMC Huymano inducido por ConA y LPS

Concentración de Composición AGP (µg/ml)	*Promedio % Inhibición*
2.00	32.31
0.20	43.28
0.02	35.41

*Los valores se describen en porcentaje de inhibición con referencia al control.

La composición AGP aislada del *Argemone mexicana* se encuentra inhibidora para la producción TNF-α inducida por mitógeno en el rango de 0.002µg/ml a 2µg/ml (Fig. 7). Ésta actividad inhibidora para la producción TNF-α por mitógeno es conocida por ser inmunosupresora y bien establecida por ser útil en el tratamiento y/o profilaxis de psoriasis. Los resultados se resumen en la Tabla VIII.

Tabla VIH

Efecto de las Composiciones AGP en la producción de TNF-a mediante PBMC Humano inducido por ConA y LPS

Concentración de Composición AGP (µg/ml)	*Promedio % Inhibición*
2.00	60.68
0.20	46.18
0.02	22.02
0.002	10.30

* Los valores se describen en porcentaje de inhibición con referencia al control.

Efecto de la Composición AGP sobre la proliferación de queratinocitos Humanos inducidos por NGF

Se compran queratinocitos de GIBCO (USA) y se mantienen en medio libre de suero-queratinocito (GIBCO, # 10744-019) complementado con factores de crecimiento. Aproximadamente, 150 µl de KGM (medio de crecimiento de queratinocito) que contiene 2000 células se agregan en cada pozo de placa de fondo plano de 96 pozos. Al siguiente día el medio se cambia con 1:2 de volumen de KGM: KBM (medio basal de queratinocito). Se agrega treinta microlitros de composición AGP diluida en cada pozo por triplicado excepto los pozos de control celular. El NGF (Factor de crecimiento de tejido nervioso) 100 ng/ml se agrega en cada pozo. El medio se remueve después de 8 días de incubación. Las células se enjuagan con PBS. Se agrega solución de lisis de desarrollo de LDH (Lactato deshidrogenasa) e incuba durante 10

min a 37° C. El OD se mide en lector ELISA en longitud de 492 nm. Se calcula el porcentaje de proliferación/ inhibición. Los resultados se resumen en la Tabla IX.

Tabla IX

Efecto de la Composición AGP en proliferación de queratinocitos Humanos inducidos por NGF

Composición AGP (µg/ml)	*Promedio % Inhibición*
40.00	79.15
8.00	74.53
1.60	71.49
0.32	66.78
0.064	55.75
0.0128	26.78
0.0025	15.36
0.0005	6.18
0.0001	3.03

*Los valores se describen en porcentaje de inhibición con referencia al control.

La composición AGP aislada del *Argemone mexicana* se encuentra inhibidor para la proliferación queratinocitos humanos inducida por NGF en el rango de 0.0001µg/ml a 40µg/ml (Figs. 8 & 9). Esta actividad inhibidora para la proliferación de queratinocitos humanos inducida por NGF se conoce por ser inmunosupresora y bien

establecida por ser útil en el tratamiento y /o profilaxis de psoriasis.

Descripción de los Métodos de Evaluación de Inmunosupresión in vivo utilizando Hipersensibilidad de Tipo Retardado en Conejillos de Indias

Éste procedimiento estándar se utiliza para la evaluación de la eficacia in vivo de la composición AGP para su capacitas de inhibir derivados de proteína purificados (PPD) inducidas por hipersensibilidad de de tipo retardado en conejillos de indias. En resumen se sensibilizan conejillos de indias con 100 µg de PPD intradérmicamente con adyuvante completo de Freund. Dos reforzadores posteriores de 100 µg de PPD con adyuvante incompatible de Freund se dan en una semana de intervalo. La composición AGP se administra oralmente una vez al día durante 28 días. Los animales se inoculan con 100 µg de PPD intradérmicamente en el día 28 y la diferencia en el control y el espesor de la piel inyectada PPD se mide después de 24 horas post inoculación con un calibre de nonio. Las diferencias de espesor de piel se calculan al sustraer el espesor de piel inyectada con salina en los mismos conejillos de indias. El porcentaje de inhibición se calcula con referencia a los animales sensibilizados con salina.

La composición AGP aislada del *Argemone mexicana* se encuentra que es inmunosupresor para conejillos de indias inoculados con PPD y sensibilizados con PPD en el rango de 20-65.89% de inhibición en la dosis de 0.10 - 100mg/kg p.o. La dosis efectiva que origina el 50% de inhibición (ED_{50}) de el grosor de piel en conejillos de indias inoculados con PPD se encuentra que es de 0.508 mg/kg p.o (Fig. 10). La propiedad inmunosupresora potente es bien establecida y es benéfica para tratamiento anti-psoriasis.

La Inmunosupresión en el modelo DTH es un modelo útil para varias enfermedades en donde se requiere Inmunosupresión, tal como psoriasis, dermatitis, escleroderma, trastornos inflamatorios y otras enfermedades autoinmunes similares a artritis psoriática, psoriasis de placa y psoriasis gotosa. Los resultados se resumen en la Tabla X.

Tabla X

Efecto de la Composición AGP en espesor de la piel en conejillos de indias inoculados con PPD

Concentración de Composición AGP mg/kg	*Porcentaje de Respuesta*
0.01	0.000
0.10	20.000

1.00	62.680
10.00	67.930
30.00	61.510
100.00	65.880

Descripción de los Métodos de Evaluación del TPA

Se aleatorizan ratones Balb/C y aclimatan en jaulas 5 días antes del experimento. Se remueve el pelo mediante el uso de una aplicación tópica de Anne French y se limpia apropiadamente. Se hacen grupos que consisten de seis ratones cada uno. Grupos de control de acetona y grupos de control TPA (12-O-tetradecanoilforbol-13-acetato) se toman juntos con el grupo de droga de ensayo. La composición AGP se disuelve en el vehículo recomendado (acetona) y los animales se dosifican oralmente durante 4 días una vez al día. Veinte microlitros de 100 nM de TPA se aplican en el segundo día en la superficie de la piel limpia y se le permite absorber. Se sacrifican los animales 72 horas después de aplicación TPA. Las piezas de piel se fijan en 10% de formalina durante 3 días. Se hacen portaobjetos histopatológicos mediante los siguientes métodos estándar. Se mide el espesor epidérmico en al menos 10 áreas diferentes al utilizar un oculómetro y los resultados se expresan en porcentaje de inhibición (Fig. 11). Los resultados se resumen en la Tabla-XI.

Tabla XI

Efecto de la Composición AGP en modelo TPA

Grupos	Dosis (mg/kg,p.o.)	Incremento en espesor epidérmico (µm)	% de inhibición
Grupo de control de acetona	-	23.08	-
Grupos de control TPA	-	52.88	0.00
Composición AGP	30.00	25.38	92.30
	10.00	29.67	77.89
	3.00	35.63	57.90
	1.00	50.08	9.37
	0.30	52.63	0.84
	0.10	52.67	0.70

*Los valores se describen en porcentaje de inhibición con referencia al grupo de control TPA.

Descripción de los Métodos de Evaluación de Inmunosupresión in vivo utilizando Ensayo de Hinchamiento de Oreja de Ratón (MEST)

Se utiliza éste procedimiento estándar para la evaluación de la eficacia in vivo de las composiciones AGP para su capacidad de inhibir la hipersensibilidad de tipo retardado inducida por DNFB en ratones [Cornacoff J. B, House R. V, Dean J. H., *Fundam. Appl. Toxicol.*, 1988, 10(1), 40; Fundam. Appl. Toxicol., 1992, 19(1), 157].

En resumen, se utilizan ratones C57BL6 para el ensayo. Se sensibilizan los ratones con 0.2% de NFB (en 1:4 de aceite de Oliva y Acetona) en el lomo de los ratones. Se hace una aplicación de tres refuerzos DNFB cada tercer día. Los ratones de inoculan con 0.2% de DNFB (en 1:4 de aceite de Oliva y Acetona) en el pabellón de la oreja. El espesor de la oreja en el centro de la oreja se mide después de 24 horas con la ayuda de calibre de nonio. El análisis se desarrolla al calcular el porcentaje de inhibición con respecto a un control negativo que se dio oralmente en diferentes dosis. Los resultados se resumen en la Tabla XII.

Tabla XII

Efecto de la Composición AGP en Ensayo de hinchamiento de Oreja de Ratón inducido por DNFB en Ratones Hembra C57/BL6

Serie No.	Tratamiento	Porcentaje de Inhibición
1	Agua Milli Q 10 ml/kg, p.o. Control negativo para composición AGP	0.00
2	2%-80, 10 ml/kg, p.o. Control negativo para Ciclosporina	0.00
3	Composición AGP - 0.1 mg/kg, p.o.	0.00
4	Composición AGP - 1 mg/kg, p.o.	18.00
5	Composición AGP - 3 mg/kg, p.o.	33.05
6	Composición AGP - 10 mg/kg, p.o.	43.62
7	Composición AGP - 30 mg/kg, p.o.	58.52
8	Composición AGP - 100 mg/kg, p.o.	69.51
9	Ciclosporina 25 mg/kg, p.o.	65.16

Datos representados como porcentaje de inhibición con cada uno de los 7-12 animales. La composición AGP se compara grupo tratado con agua Milli Q y la ciclosporina se compara con animales tratados con 2% Tween 80

La composición AGP de las hojas y/p tallos de *Argemone mexicana* se encuentra que son inmunosupresores para ratones C57BL6 sensibilizados con DNFB. El ED_{50} para la composición AGP se determina que es 6.43 mg/kg (Fig. 12). La propiedad inmunosupresora potente es bien establecida y benéfica para el tratamiento o profilaxis de psoriasis. La Inmunosupresión en el modelo MEST es útil en la evaluación del efecto para varias enfermedades en donde se requiere Inmunosupresión, tal como psoriasis, dermatitis, escleroderma, trastornos inflamatorios y

otras enfermedades autoinmunes como artritis psoriática, psoriasis de placa, psoriasis gotosa, artritis reumatoide, enfermedad de Crohn, esclerosis múltiple, espondilitis anquilosante, lupus eritematoso sistémico, síndrome de Sjogren, alergias similares a asma, enfermedad pulmonar obstructiva crónica y condiciones relacionadas como eczema, dermatitis atópica.

La invención se ilustra adicionalmente por los siguientes ejemplos no limitantes.

Ejemplo 1

Aislamiento de la composición AGP, AGP-HM y AGP-LM

Se muelen 10 Kg de hojas frescas de *Argemone mexicana* y se extraen con agua desmineralizada (15 litros). La suspensión se centrifuga y el extracto acuoso se concentra por debajo de 40° C bajo vacío. El material concentrado se liofiliza para dar 0.54 Kg de sólido seco.

500 g del extracto acuoso seco se disuelve en 6 litros de agua, se centrifuga y se decanta. El supernadante se carga en una columna de intercambio de catión (5 litros) en la taza de 10 ml/min. Se recolecta el eluado (7.5 litros), se concentra (2.5

litros) y se carga en una columna de intercambio de anión (5 litros). El eluado (3 litros) se concentra aproximadamente 1.5 litros. El experimento se reparte con otro 0.5 kg del material (extracto acuoso). Los eluados combinados (1.5 litros) cada uno se mezclan y concentran a 2.5 litros y se carga en una columna de intercambio de cationes. El eluado (3 litros) se concentra hasta 1.2 litros y se carga en una columna de intercambio de anión. El eluado (1.5 litros) se liofiliza para dar 42.39 g de un polvo.

42 g del polvo anterior, se disuelve en 600 ml de agua y se particiona entre n-butanol (3 X 400 ml) y agua. Se desecha la capa de n- butanol. Se vierten 3 litros de metanol en la capa acuosa (600ml) cuando se precipita un sólido. La solución se centrifuga y el supernadante se decanta. El supernadante se concentra a un volumen de 300ml. Ca. El metanol (1 litro) se vierte de nuevo para precipitar los insolubles de metanol. La solución se centrifuga y el supernadante se decanta. El sólido precipitado de ambos experimentos se liofiliza (8.75 g). Los materiales secos se disuelven en agua, y se someten a columna XAD-2, dos veces. El eluado de agua se liofiliza para dar 6.10 g de una composición AGP. Éste se somete adicionalmente a cromatografía de sefacril para dar 5 mg de AGP-HM y 10.3 mg de AGP-LM.

Ejemplo 2

Aislamiento de la composición AGP, AGP-HM y AGP-LM

22 Kg de hojas frescas de *Argemone mexicana* se muelen n y se extraen con agua desmineralizada (36 litros). La suspensión se centrifuga y el extracto acuoso se concentra por debajo de 40° C bajo vacío. El material concentrado se liofiliza para dar 1.25 Kg de sólido seco.

1.2 kg de extracto acuoso seco se disuelve en 12 litros de agua, se centrifuga y se decanta. El supernadante se carga en una columna de intercambio de cationes (5 litros) en la proporción de 10 ml/min. El eluado (15.5 litros) se recolecta, se concentra, (5.5 litros) y se carga en una columna de intercambio de anión (5 litros).El eluado (6.25 litros) se concentra a aproximadamente 3.2 litros.

El eluado (3.2 litros) se concentra a 2.6 litros y se carga en una columna de intercambio de catión. El eluado a (4 litros) se concentra hasta 1.5 litros y se carga en una columna de intercambio de anión. El eluado (1.6 litros) se liofiliza para dar 54 g de un polvo.

40 g del anterior polvo se disuelve en 700 ml de agua y se particiona entre n-butanol (3 X 500 ml) y agua. Se desecha la

capa de n-butanol. Se vierten 4 litros de metanol en la capa acuosa (675 ml) cuando un precipitado sólido sale. La solución se centrifuga y el supernadante se decanta. El supernadante se concentra a 400 ml. El metanol (3 litros) se vierte de nuevo para precipitar los insolubles de metanol. La solución se centrifuga y el supernadante se decanta. Se liofilizan los sólidos precipitados (7.3 g). El material seco así obtenido se disuelve en agua, y se somete a columna XAD-2, dos veces. El eluado de agua se liofiliza para dar 5.5 g de composición AGP. Esto se somete adicionalmente a cromatografía sefacril para dar 4.5 mg de AGP-HM 12.4 mg de AGP-LM.

Ejemplo 3

Tabla XIII

Fórmula Unitaria para una Formulación de Cápsula que Comprende el AGP de la Presente Invención y Portadores Farmacéuticamente Aceptables

Serie No.	Ingrediente	Resistencia mg/cápsula	
01	AGP	50	200
02	Celulosa Microcristalina USNF (Avicel PH 102)	39	39
03	Croscarmelosa de Sodio (Ac-Di-SOL)	10	10
04	Dióxido de Silicio Coloidal USNF (Aerosil 200)	0.5	0.5

05	Estearato de Magnesio USNF	0.5	0.5
	Total	100	250
06	Cápsula de gelatina dura	1 (tamaño "2" con cuerpo naranja y tapa naranja)	1 (tamaño "00" con cuerpo naranja y tapa roja)

Los ingredientes mencionado anteriormente dados en la Tabla XIII se pueden mezclar hasta uniformidad y luego llenar en cápsula de gelatina dura del tamaño "00". El área de procesamiento se mantiene idealmente en 40±5% RH a 18-22E C.

Un proceso típico para la preparación de la composición de la invención se ilustra adelante:

El ingrediente activo, Celulosa Microcristalina, Croscarmelosa de Sodio, Estearato de Magnesio y dióxido de silicio coloidal se mezclan hasta uniformidad. Se llena en cápsulas de gelatina dura del tamaño de "00". El área de procesamiento de mantiene idealmente en 40±5% RH a 18EC a 22EC.

Cuando se administra una aplicación tópica, la cantidad de planta *Argemone mexicana* varia de 0.1% a 10% en peso del extracto.

Una fórmula unitaria para un ungüento para aplicación tópica que comprende el AGP y el portador se resumen en la Tabla XIV.

Tabla-XIV

Fórmula Unitaria para un Ungüento para Aplicación Tópica que Comprende el AGP de la Presente Invención y Portadores Farmacéuticamente

Serie No.	***Ingrediente***	***Cantidad***
01	AGP	1.0 gm
02	Agua	100 ml
03	Hidroxipropil MetilCelulosa	4.0 gm

Un proceso típico para la preparación de un ungüento para aplicación tópica comprende mezclar lentamente todos los

ingredientes hasta que se obtiene un gel liso por métodos convencionales y llenar tubos con éste

REIVINDICACIONES

1. Una composición de proteína-arabinogalactan (AGP) purificada, que tiene un peso molecular promedio que varía entre 10 KD a 150 KD, aislado de las hojas y/o tallos de la planta *Argemone mexicana* obtenible mediante un proceso que comprende las etapas de:

i) extraer una parte en peso de las hojas y/o tallos de la planta *Argemone mexicana* con 1 a 10 partes en peso de agua, alcohol C_{1-3} o mezclas de éstos para obtener un extracto acuoso; y concentrar parcial o completamente o liofilizar el extracto;

ii) someter el extracto acuoso, extracto acuoso parcialmente concentrado o una solución acuosa del extracto liofilizado o completamente concentrado como se obtiene en la etapa i) sucesivamente para cromatografía de intercambio de ión para obtener un extracto acuoso neutro;

iii) fraccionar el extracto acuoso neutro obtenido en la etapa ii) con n-butanol y separar las fases acuosa y la fase n-butanol;

iv) mezclar y agitar las fases acuosas obtenidas en la etapa iii) con metanol o etanol, y aislar los sólidos precipitados para obtener una fracción insoluble; y

v) someter la fracción insoluble obtenida en la etapa iv) para cromatografía de gel sucesiva y cromatografía de exclusión de tamaño para obtener la composición de proteína-arabinogalactán (AGP) purificada.

2. La composición de acuerdo con la reivindicación 1, en donde el alcohol C_{1-3} se selecciona de metanol, etanol, 1-propanol o 2-propanol.

3. La composición de acuerdo con la reivindicación 1, en donde la cromatografía de intercambio de ión es cromatografía de intercambio de catión seguida por cromatografía de intercambio de anión.

4. La composición de acuerdo con la reivindicación 1, en donde la cromatografía de intercambio de ión es cromatografía de intercambio de anión seguido por cromatografía de intercambio de catión.

5. La composición de acuerdo con la reivindicación 3 o 4, en donde la cromatografía de intercambio de catión se lleva a cabo sobre intercambiadores de ácido fuerte de poliestireno sulfonatado o intercambiadores de catión de ácido débil del tipo ácido carboxílico.

6. La composición de acuerdo con la reivindicación 3 o 4, en donde la cromatografía de intercambio de anión se lleva a cabo sobre intercambiadores de anión de base débil del tipo amina alifática o intercambiadores de anión de base fuerte.

7. La composición de acuerdo con la reivindicación 1, sustancialmente libre de otros alcaloides, flavonoides, aminoácidos, ácidos orgánicos, ácidos grasos, y otros compuestos presentes en las hojas y/o tallos de la planta *Argemone mexicana*.

8 La composición de acuerdo con la reivindicación 1, que comprende galactopiranosa 6-ligada y galactopiranosa 3,6-ligada como la estructura y los residuos arabinofuranosilo, arabinopiranosilo, ramnopiranosilo, glucopiranosilo y galactopiranosilo en las posiciones terminales, y los residuos ramnopiranosilo 3-ligado□□-4-ligado□, mannopiranosilo 2-ligado, glucopiranosilo 2-ligado, galactopiranosilo 3-ligado, 4-ligado, glucopiranosilo 6-ligado, 3,6-ligado, xilopiranosilo 4-ligado, junto con ácido metil urónico.

9. La composición de acuerdo con la reivindicación 1, que se constituye de 45-98% en peso de carbohidratos.

10. La composición de proteína-arabinogalactán (AGP) purificada de acuerdo con la reivindicación 1, que se constituye de 2-20% en peso de proteínas.

11. Un método para aislar la composición de proteína-arabinogalactán (AGP), que comprende las etapas de:

i) extraer una parte en peso de las hojas y/o tallos de la planta *Argemone mexicana* con 1 a 10 partes en peso de agua, un alcohol C_{1-3} o mezclas de éstos para obtener un extracto acuoso y concentrar completa o parcialmente o liofilizar el extracto;

ii) someter el extracto acuoso, extracto acuoso concentrado parcialmente o una solución acuosa del extracto concentrado/liofilizado completamente como se obtiene en la etapa i) sucesivamente para cromatografía de intercambio de ión para obtener un extracto acuoso neutro;

iii) fraccionar el extracto acuoso neutro obtenido en la etapa ii) con n-butanol y separar la fase acuosa y la fase n-butanol;

iv) mezclar y agitar las fases acuosas obtenidas en la etapa iii) con metanol o etanol, y aislar los sólidos precipitados para obtener una fracción insoluble; y

v) someter la fracción insoluble obtenida en la etapa iv) a cromatografía de gel sucesiva y cromatografía de exclusión de tamaño para obtener composición de proteína-arabinogalactán (AGP) purificada.

12. El método de acuerdo con la reivindicación 11, en donde el alcohol C_{1-3} se selecciona de metanol, etanol, 1-propanol o 2-propanol.

13. El método de acuerdo con la reivindicación 11, en donde la cromatografía de intercambio de ión es cromatografía de intercambio de catión seguida por cromatografía de intercambio de anión.

14. El método de acuerdo con la reivindicación 11, en donde la cromatografía de intercambio de ión es cromatografía de intercambio de anión seguida por cromatografía de intercambio de catión.

15. El método de acuerdo con la reivindicación 13 o 14, en donde la cromatografía de intercambio de catión se lleva a cabo sobre intercambiadores de catión de ácido fuerte de poliestireno sulfonatado o intercambiadores de catión de ácido débil del tipo ácido carboxílico.

16. El método de acuerdo con la reivindicación 13 o 14, en donde la cromatografía de intercambio de anión se lleva a cabo sobre intercambiadores de anión de base débil del tipo amina alifática o intercambiadores de anión de base fuerte.

17. El método de acuerdo con la reivindicación 11, en donde la cromatografía de gel se lleva a cabo sobre un adsorbente polimérico de Amberlita.

18. El método de acuerdo con la reivindicación 11, en donde la cromatografía de exclusión de tamaño se lleva a cabo sobre sefacril.

19. El método de acuerdo con la reivindicación 11, en donde la cromatografía de gel se lleva a cabo sobre unos adsorbentes poliméricos de Amberlita seleccionados de XAD-2, XAD-4 y XAD-7.

20. El método de acuerdo con la reivindicación 11, en donde la cromatografía de exclusión de tamaño se lleva a cabo sobre sefacril seleccionado de sefacril S-100, sefacril S-200 HR, y sefacril S-300 HR.

21. Una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la proteína-arabinogalactán (AGP) purificada como se reivindica en una cualquiera de las reivindicaciones 1 a 10 y un excipiente farmacéuticamente aceptable.

22. La composición de acuerdo con la reivindicación 21, que comprende adicionalmente al menos unos llenadores compatibles no tóxicos, ligadores, desintegrantes, amortiguadores, preservativos, antioxidantes, lubricantes, o agentes saborizantes.

23. La composición farmacéutica de acuerdo con la reivindicación 21, en donde el excipiente farmacéuticamente aceptable se selecciona de azúcares, almidones, celulosa, y sus derivados fosfatos de calcio; sulfato de sodio; sulfato de calcio; polivinilpirrolidona; alcohol polivinílico; ácido esteárico; aceites vegetales; tensoactivos no iónicos, catiónicos y aniónicos; polímero de etilenglicol; β-ciclodextrina; alcoholes grasos; o sólido de cereal hidrolizado.

24. Una proteína-arabinogalactán (AGP) purificada que tiene un peso molecular promedio que varía entre 10 KD a 150

KD, aislado de las hojas y/o tallos de la planta *Argemone mexicana*.

25. Una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la proteína-arabinogalactán (AGP) purificada de la reivindicación 59 y un excipiente farmacéuticamente aceptable

26. Una proteína-arabinogalactán (AGP) purificada que tiene un peso molecular promedio que varía entre 10 KD a 150 KD, aislado de las hojas y/o tallos de la planta *Argemone mexicana*.

27. Uso de una proteína-arabinogalactán (AGP) purificada como se reivindica en una cualquiera de las reivindicaciones 1 a 10, 24 o 26 en la preparación de un medicamento para el tratamiento de la psoriasis, trastorno inflamatorio, enfermedad autoinmune, alergia, eczema o dermatitis atópica, o deterioro de piel en mamíferos.

28 Uso de una composición como se reivindica en una cualquiera de las reivindicaciones 21 a 23 o 25 en la preparación de un medicamento para el tratamiento de psoriasis, trastorno inflamatorio, enfermedad autoinmune,

alergia, eczema o dermatitis atópica, o deterioro de piel en mamíferos.

29. Uso de acuerdo con las reivindicaciones 24 o 25, en donde el trastorno se selecciona del grupo que consiste de dermatitis; escleroderma; eczema; artritis psoriática, artritis reumatoide, enfermedad de Crohn, esclerosis múltiple, enfermedad del intestino irritable, espondilitis anquilosante, lupus eritematoso sistémico, síndrome de Sjogren, y dermatitis atópica.

30. Uso de acuerdo con las reivindicaciones 24 o 25 en donde dicha alergia es asma o enfermedad pulmonar obstructiva crónica.

31. Uso de acuerdo con las reivindicaciones 24 o 25 en donde dicha psoriasis se selecciona del grupo que consiste de psoriasis de placa, psoriasis gotosa, psoriasis pustulosa y psoriasis de las uñas.

RESUMEN

Se describe una composición de proteína-arabinogalactán (AGP) purificada aislada a través de un método selectivo de las hojas y/o tallos de la planta *Argemone mexicana*. También se describe una composición de proteína-arabinogalactán (AGP) purificada aislada de las hojas y/o tallos de la planta *Argemone mexicana*, que tiene uno o más de los siguientes efectos: inmunosupresión, inhibición de linfoproliferación, modulación de citoquina tal como inhibición IL-2, inhibición IFN-γ e inducción IL-10; inhibición de proliferación de queratinocitos, actividad queratolítica, y actividad inhibidora en ensayo de hinchamiento de oreja de ratón (MEST).

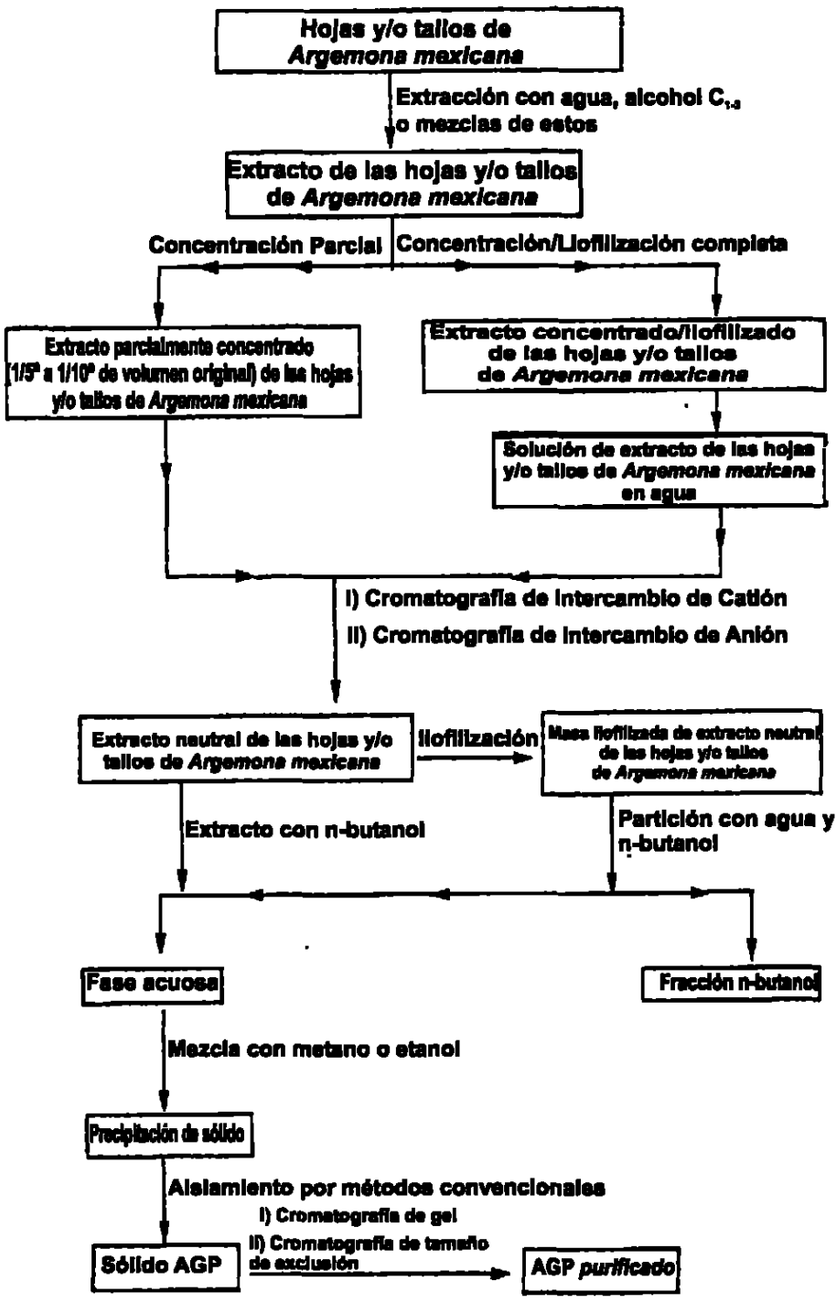

Fig. 1: El Método Selectivo para Aislamiento de Composición de Proteína-Arabinogalactan (AGP) Purificada como por la Presente Invención

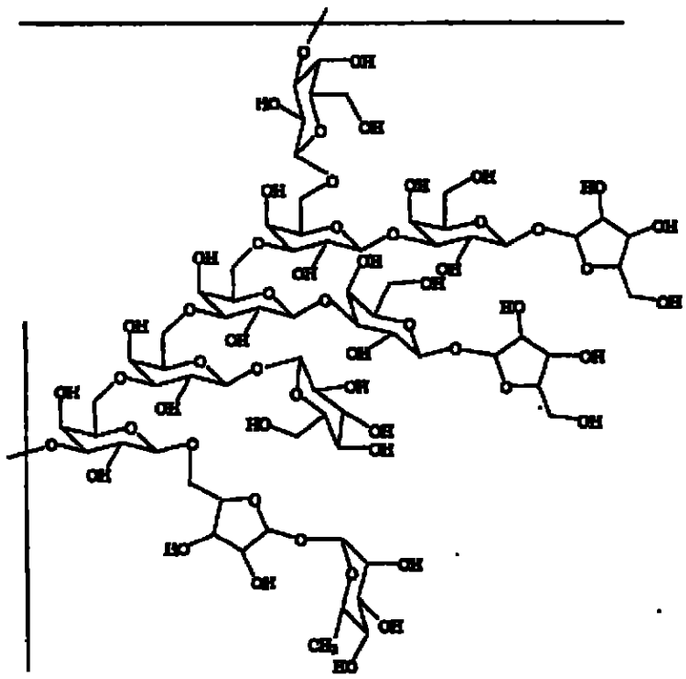

Fig. 2 Estructura Propuesta de la Composición AGP

Efecto de la Composición AGP en Inducción IL-10

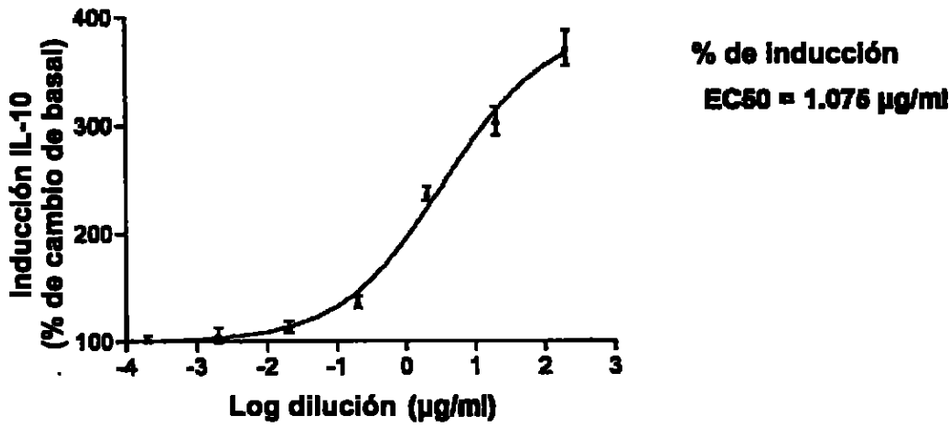

Fig. 3: Efecto de la Composición AGP en Inducción IL-10

Efecto de la Composición AGP en la Inhibición IL-2

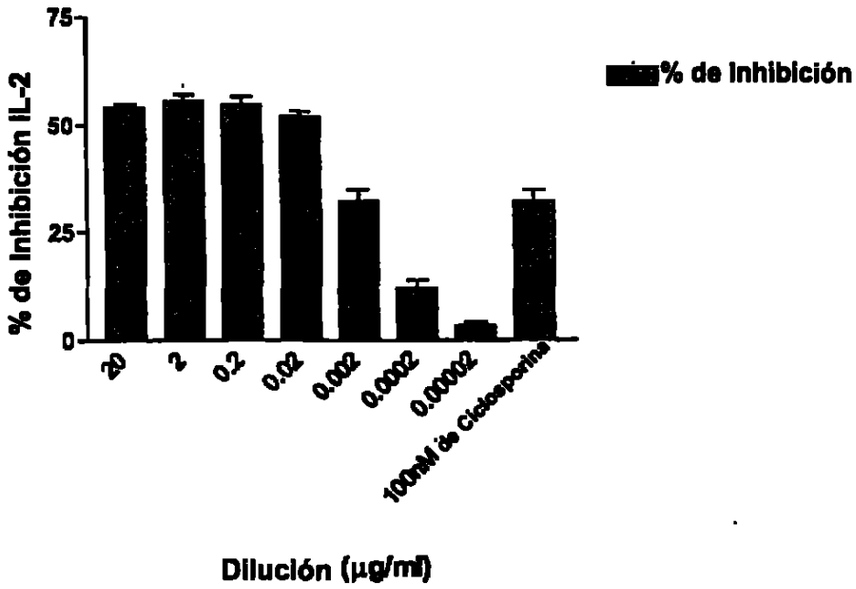

Fig. 4: Efecto de la Composición AGP en la Inhibición IL-2

Efecto de la Composición AGP en la Inhibición IFN-γ Humana

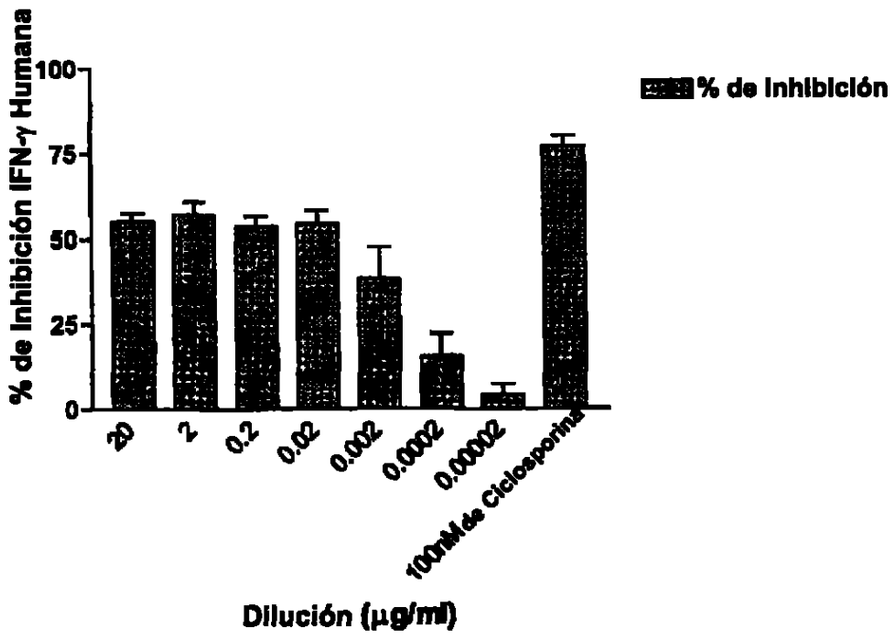

Fig. 5: Efecto de la Composición AGP en la Inhibición IFN-γ Humana

Efecto de la Composición AGP en la Inhibición GMCSF

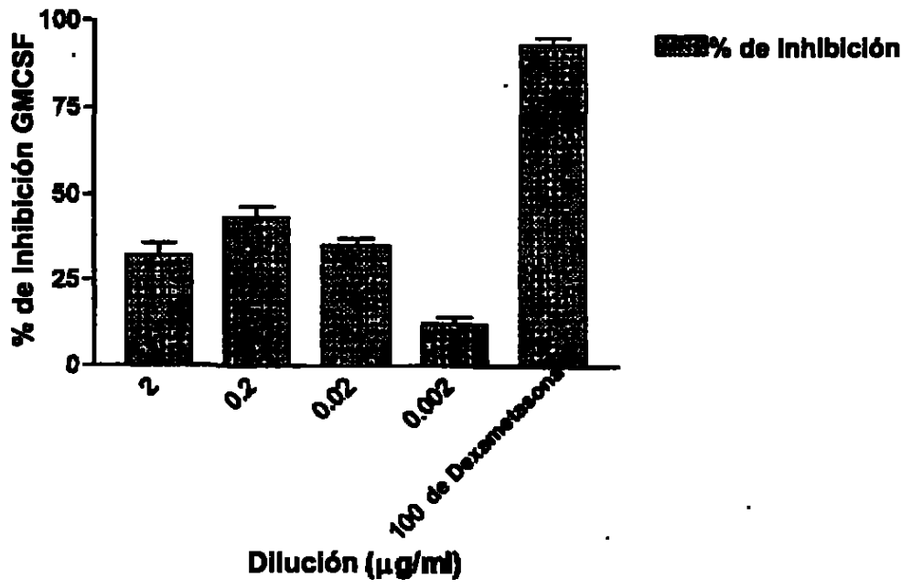

Fig. 6: Efecto de la Composición AGP en la Inhibición GMCSF

Efecto de la Composición AGP en la Inhibición TNF-α Humana

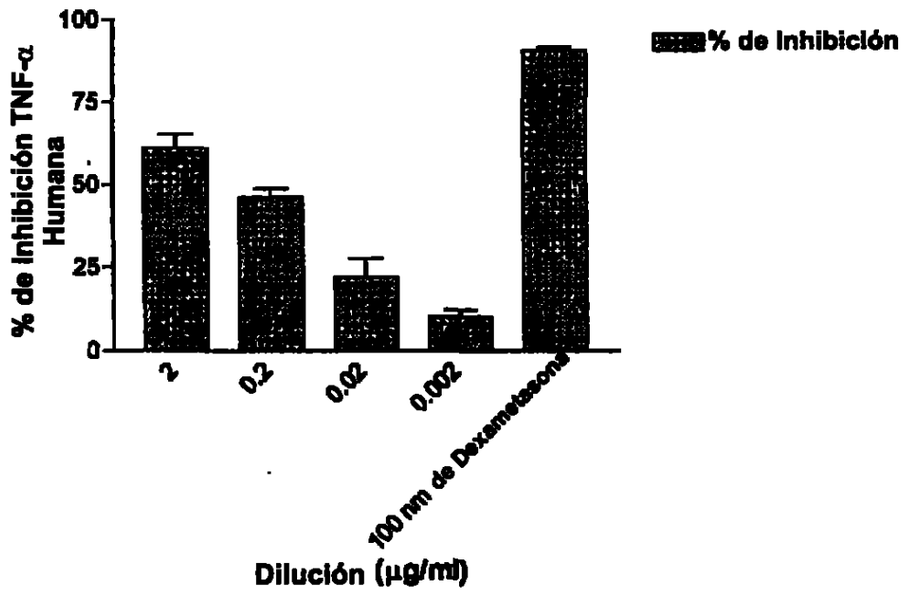

Fig. 7: Efecto de la Composición AGP en la Inhibición TNF-α Humana

Efecto de la Composición AGP en la Proliferación de Queratinocito Humano Inducido por NGF

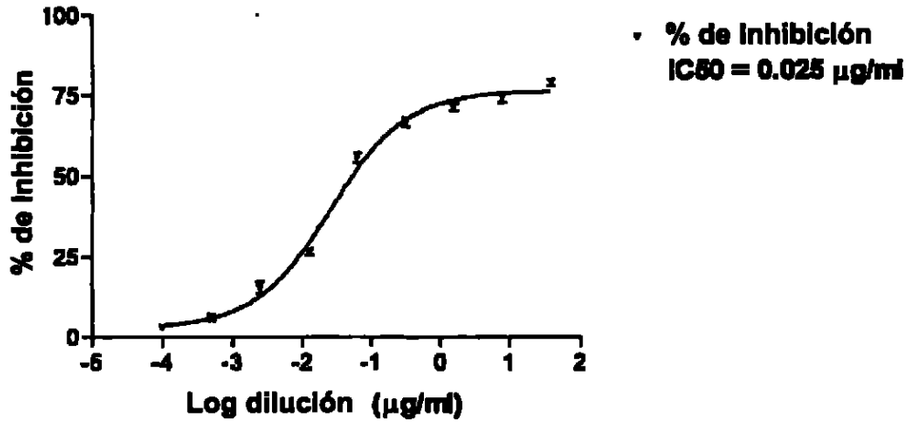

Fig. 8: Efecto de la Composición AGP en Proliferación de Queratinocito Humano Inducido por NGF

Efecto de la Composición AGP en la Proliferación de Queratinocito Humano Inducido por NGF

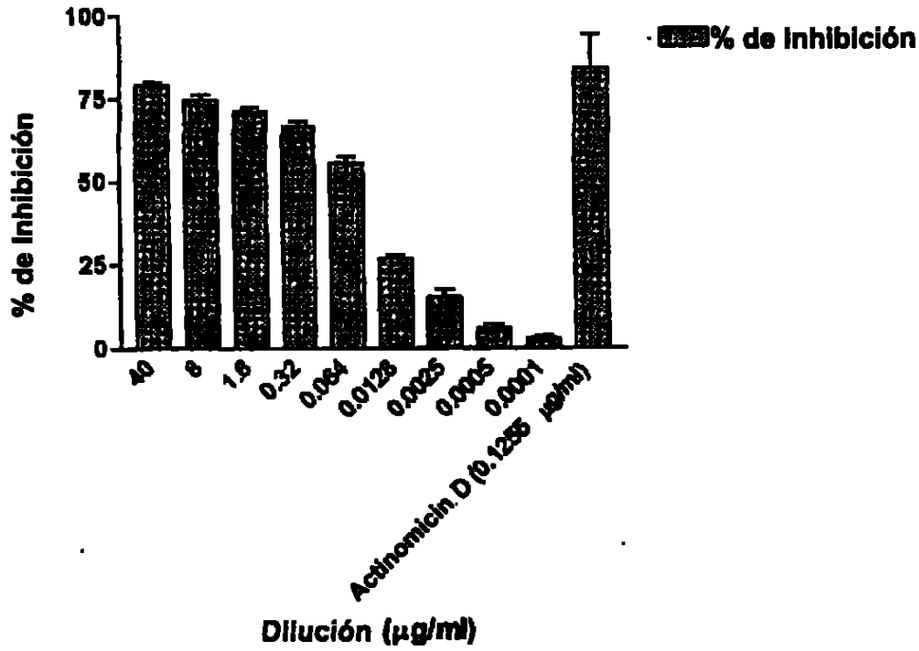

Fig. 9: Efecto de la Composición AGP en la Proliferación de Queratinocito Humano Inducido por NGF

Efecto de la Composición AGP en espesor de piel en DTH en conejillos de indias

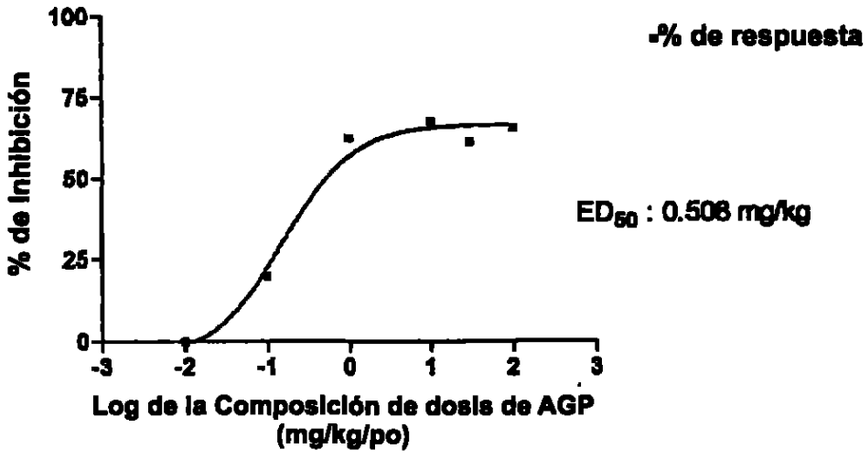

Fig. 10: Efecto de la Composición AGP en Espesor de Piel en Conejillos de Indias Inoculados con PPD

Efecto de la Composición AGP en la Inhibición de Espesor Epidérmico Inducido por TPA en ratones balb/c

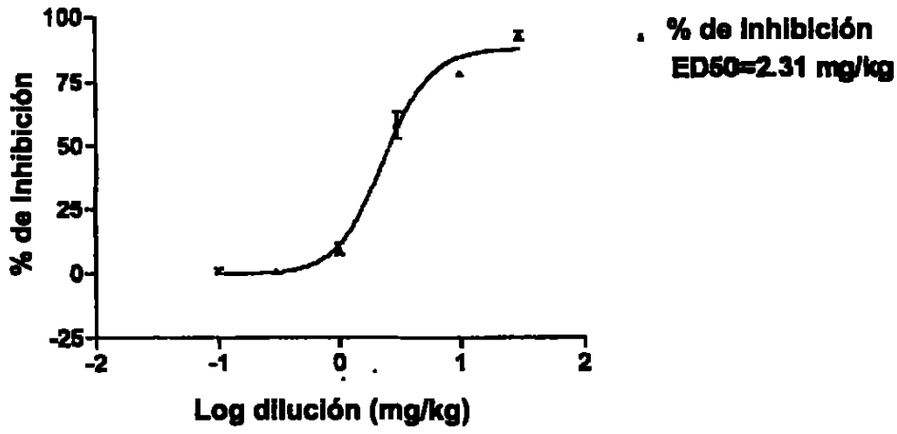

Fig. 11: Efecto de la Composición AGP en Espesor Epidérmico Inducido por TPA en ratones balb/c

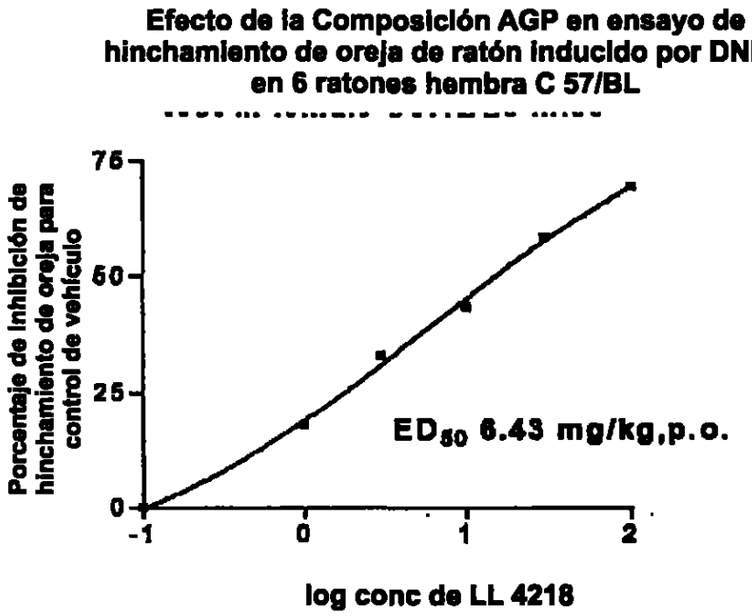

Fig. 12 Efecto de la Composición AGP en Ensayo de Hinchamiento de Oreja de Ratón Inducido por DNFB en 6 Ratones Hembra C 57/BL

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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E-556 Greater Kailash II
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INDE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	08.08.2006
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
LUP-PSOR-II	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN2005/000132	28.04.2005	01.09.2004

Applicant
LUPIN LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

   The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

   Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

   For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

   The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
LUP-PSOR-II		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/IN2005/000132	26.04.2005	01.09.2004

International Patent Classification (IPC) or national classification and IPC

INV. C07K14/415

Applicant

LUPIN LIMITED et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (Indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in calectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the International application
☒	Box No. VIII	Certain observations on the International application

Date of submission of the demand	Date of completion of this report
28.03.2006	08.08.2006

Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bulcao de Melo Barre Telephone No. +49 89 2399-8472

Form PCT/IPEA/409 (Cover Sheet) (April 2005)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IN2005/000132

179

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1 (b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-45	as originally filed
Claims, Numbers	
1-4, 12-31	as originally filed
Drawings, Sheets	
1/12-12/12	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 5-11

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 5-11

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4, 12-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-4, 12-31
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-4, 12-31
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Reference is made to the following document:

D1: US 2003/194456

SECTION V

2. Novelty (Article 33(2) PCT)

The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations (Rule 64 (1)-(3) PCT).
Therefore, in view of such prior art the subject-matter of claims 1-4 and 12-31 of the present application has to be regarded as being new (Article 33 (2) PCT).

3. Inventive Step (Article 33 (3) PCT)

The closest prior art to evaluate the inventiveness of the present application is document D1, which discloses a process for obtaining an extract from the leaves and/or stems of the *Argemone mexicana* plant and fractionation of the extract with n-butanol and methanol to give the respective n-butanol-soluble, methanol-soluble and methanol-insoluble fractions.

The present inventors have found that the methanol-insoluble fraction is constituted of arabinogalactan proteins (AGPs) in mixture with other components and provide a selective method for the isolation of AGPs in purified form from the leaves and/or stems of the *Argemone mexicana* plant.

The selective method of the present application differs from the method of D1 in that it further comprises: a) subjecting the initial aqueous extract to cation and anion exchange chromatography for removal of the basic and acid components and b) subjecting the methanol or ethanol-insoluble fraction to gel chromatography and size exclusion chromatography to obtain purified AGP.
This has not been suggested in the prior art.

The purified AGP composition obtainable by the method of the present application exhibits vastly superior antipsoriatic activity and other useful immunological and pharmacological activities compared to the extract and fractions of the leaves and/or stems of the plant *Argemone mexicana* obtainable by the method of D1.
A pharmaceutical composition comprising said purified AGP composition provides a safe, cost-effective and well-tolerated treatment for psoriasis and other diseases.

Therefore, an inventive step can be acknowledged for the subject-matter of the present application.

4. Industrial Applicability (Article 33(4) PCT)

The subject-matter of present claims 1-4 and 12-31 is susceptible of industrial applicability as defined in Article 33 (4) PCT.

SECTION VIII

5. The present application does not satisfy the criterion set forth in Article 6 PCT because the following claims are not clear.

5.1. Claim 24 refers to a purified Arabinogalactan protein (AGP).
However, the method of the present application provides a purified Arabinogalactan protein (AGP) composition and not a purified protein.
The description refers that the purified Arabinogalactan protein (AGP) composition of the present invention is constituted of several fractions of differing molecular weights, wherein such fractions have average molecular weights as high as 115 to 150 KD and as low as 10 to 15 KD (page 17, lines 27-30). The description also indicates that the components of the AGP composition have an average molecular weight range between 10 KD to 150 KD (page 17, lines 5-6).
Therefore, claim 24 should be reworded. It should be directed to a purified Arabinogalactan protein (AGP) composition obtainable by the method of the present invention and should reflect the above passages of the description.

Moreover, claim 24 lacks clarity because the method used in the determination of th molecular weight is not indicated.

5.2. The subject-matter of **claims 24 and 26** appears to be the same.
Therefore, one of said claims is superfluous and should be deleted.

5.3. The dependency of **claim 25** on claim 59 appears to be incorrect.
It appears that said claim should depend on claim 24.

5.4. The dependencies of **claims 29-31** on claims 24 or 25 appear to be incorrect.
It appears that said claims should depend on claims 27 or 28.

********** * **********

6. In case of filling amended claims, the applicant is requested to take account of the above comments.

The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed (**Article 34 (2)(b) PCT** and **Rule 70.2 (c) PCT**).

In order to facilitate the examination of the conformity of the amended application with the requirements of **Article 34 (2)(b) PCT**, the Applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

SUBRAMANIAM, Hariharan
Subramaniam, Natarej & Associates
E-556 Greater Kailash II
New Delhi 110 048
INDE

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	18-04-2006
Applicant's or agent's file reference: LUP-PSOR-II	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN2005/000132	26/04/2005	01/09/2004

Applicant: LUPIN LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

28/03/2006

2. This date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is after the expiration of 19 months from the priority date. Consequently, in respect of some Offices, the demand does not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)) and the acts for entry into the national phase must therefore be performed within 20 months from the priority date (or later in some Offices). However, in respect of some other Offices, the time limit of 30 months (or later) may nevertheless apply. See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	MORENO R A Tel. (+49-89) 2399-2658

Form PCT/IPEA/402 (April 2002; reprint January 2004) (11/04/2006)

Europäisches Patentamt · European Patent Office · Office européen des brevets

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556 Greater Kailash II
New Delhi 110 048
INDE

NOTIFICATION CONCERNING PAYMENT OF THE PRELIMINARY EXAMINATION AND HANDLING FEES

(PCT Rules 57 and 58 and Administrative Instructions, Section 615)

In advance by facsimile

Date of mailing *(day/month/year)*	18-04-2006
Applicant's or agent's file reference: LUP-PSOR-II	PAYMENT DUE: see item 3 for time limit
International application No.: PCT/IN2005/000132	International filing date *(day/month/year)*: 26/04/2005
Applicant: LUPIN LIMITED et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority has received:

☐ the payment of all the prescribed fees and ☐ an overpayment, which will be refunded in due course.

☒ no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balances due, as summarized under item 2, within the time limit indicated under item 3.

2. Fees and payment calculation:

Preliminary examination fee	EUR 1595,00 [P]				
Handling fee *	+ EUR 129,00 [H]				
Total fees payable	= EUR 1724,00	-	none (Amount paid)	=	EUR 1724,00 (Balance)

* *Applicants from certain States are entitled to a reduction of 75% of the handling fee. When the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee. See Notes to the Fee Calculation Sheet annexed to the Demand Form. PCT/IPEA/401, for details.*

3. Time limit for payment and amount payable (Rules 57.3 and 58.1 (b)):

☐ within ONE MONTH from the date on which the demand was submitted (see below), in which case the amount payable is the amount applicable on that date of submittal:

_____

☐ within ONE MONTH from the date of receipt (see below) of the demand by this Authority (where the demand was transmitted to this Authority under rule 59(3)), in which case the amount payable is the amount applicable on that date of receipt:

_____

✗ within ONE MONTH from the above date of mailing: 18-04-2006

Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer MORENO R A Tel. (+49-89) 2399-2658

Form PCT/IPEA/403 (July 1998) P2045S (11/04/2006)

Europäisches Patentamt · European Patent Office · Office européen des brevets

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference: LUP-PSOR-II
International application No.: PCT/IN05/00132	International filing date (day/month/year): 26 April 2005 (26.04.2005)	(Earliest) Priority date (day/month/year): 01 September 2004 (01.09.2004)

Title of invention:
A PURIFIED ARABINOGALACTAN-PROTEIN (AGP) COMPOSITION

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	Telephone No.: + 91 20 5126161
LUPIN LIMITED 159, CST Road, Kalina, Santacruz (East) Mumbai - 400 098 Maharashtra, India (applicant for all designated states except U.S.A.)	Facsimile No.: + 91 20 5126178
	Teleprinter No.:
	Applicant's registration No. with the Office:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IN	IN

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
ARORA, Sudershan Kumar
Lupin Limited
159, CST Road, Kalina, Santacruz (East)
Mumbai - 400 098
Maharashtra, India
(inventor and applicant for U.S.A. only)

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IN	IN

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
SRIVASTAVA, Vandita
Lupin Limited
159, CST Road, Kalina, Santacruz (East)
Mumbai - 400 098
Maharashtra, India
(inventor and applicant for U.S.A. only)

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IN	IN

☒ Further applicants are indicated on a continuation sheet.

Sheet No. 2

International application No.
PCT/IN05/00132

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WALUNJ, Sameer Shankar
Lupin Limited
159, CST Road, Kalina, Santacruz (East)
Mumbai - 400 098
Maharashtra, India
(Inventor and applicant for U.S.A. only)

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
IN	IN

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/IN05/00132

Box No. III AGENT R COMMON REPRESENTATIVE; R ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SUBRAMANIAM, Hariharan; NATARAJ, Guruswamy;
AHUJA, Shalina; PRITHVIRAJ, Susheela
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi-110 048, INDIA

Telephone No.
+91 11 2921 5603

Facsimile No.
+91 11 2922 6005

Telaprinter No.

Agent's registration No. with the Office
IN/PA-93

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☒ as originally filed
☐ as amended under Article 34

the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☐ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as received.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of the applicable time limit under Rule 69.1(d).

4. ☐ The applicant expressly wishes the international preliminary examination to start earlier than at the expiration of the applicable time limit under Rule 54*bis*.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

International application No.
PCT/IN05/00132

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	: 1	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate power of attorney
3. ☐ original general power of attorney
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☐ sequence listing in computer readable form
7. ☐ tables in computer readable form related to a sequence listing
8. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

G. Nataraj
Patent Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
   ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.
6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54*bis*.1(a) and item 7 or 8, below, does not apply.
7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54*bis*.1(a) as extended by virtue of Rule 80.5.
8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54*bis*.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/IN05/00132	For International Preliminary Examining Authority use only
Applicant's or agent's file reference LUP-PSOR-II	Date stamp of the IPEA

Applicant

LUPIN LIMITED

CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee	EUR 1530.00 P	
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	EUR 129.00 H	
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	EUR 1659.00 TOTAL	

MODE OF PAYMENT

- ☐ authorization to charge deposit account with the IPEA (see below)
- ☐ cash
- ☐ cheque
- ☐ revenue stamps
- ☐ postal money order
- ☐ coupons
- ☐ bank draft
- ☐ other *(specify)*:

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all IPEAs)

IPEA/ _____

☐ Authorization to charge the total fees indicated above.

Deposit Account No.: _____

☐ *(This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

Date: _____

Name: _____

Signature: _____

See Notes to the fee calculation sheet

PATENT CO PERATI N TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
SUBRAMANIAM, NATARAJ & ASSOCIATES
Attn. Subramaniam, Hariharan
E 556 Greater Kailash II
New Delhi 110 048
INDIA

Received with Thanks
26 DEC 2005
SN&A

Applicant's or agent's file reference: LUP-PSOR-II	Date of mailing (day/month/year): 23/12/2005
	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No.: PCT/IN2005/000132	International filing date (day/month/year): 26/04/2005
Applicant: LUPIN LIMITED	

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Christian Pozzi

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

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The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

INTERNATIONAL SEARCH REPORT

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International Application No
PCT/IN2005/000132

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/415 A61K35/78 B01D15/26

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)
C07K A61K B01D

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, WPI Data, PAJ, BIOSIS, EMBASE, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/194456 A1 (ARORA SUDERSHAN KUMAR ET AL) 16 October 2003 (2003-10-16) cited in the application *Whole document, in particular: Abstract, paragraphs 100-131, example V and claims*	1-4, 12-31
A	PATIL M B ET AL: "PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND WOUND HEALING ACTIVITY OF THE LEAVES OF ARGEMONE MEXICANA LINN. (PAPAVERACEAE)" INDIAN DRUGS, vol. 38, no. 6, June 2001 (2001-06), pages 288-293, XP009014849 *Whole document, in particular: Abstract*	1-4, 12-31

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document 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
7 December 2005	23/12/2005

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	BULCAO DE MELO BARRE

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHOWALTER A M: "Arabinogalactan-proteins: Structure, expression and function" CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, vol. 58, no. 10, September 2001 (2001-09), pages 1399-1417, XP002357941 ISSN: 1420-682X *whole document, in particular: Abstract; page 1404: left hand column, last paragraph-right hand column, first paragraph; page 1413, left hand column, second paragraph; figure 2 and tables 2 and 3*	1-4, 12-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2005/000132

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5-11
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 5-11

Claims 5 to 11 have not been filed

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATI NAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IN2005/000132

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003194456 A1	16-10-2003	AU 2003222436 A1	24-07-2003
		CA 2471490 A1	17-07-2003
		EP 1467745 A2	20-10-2004
		WO 03057133 A2	17-07-2003
		JP 2005519051 T	30-06-2005
		ZA 200405439 A	08-07-2005

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN2005/000132	26.04.2005	01.09.2004

International Patent Classification (IPC) or both national classification and IPC
C07K14/415, A61K35/78, B01D15/26

Applicant
LUPIN LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

BULCAO DE MELO BARRE

Telephone No. +49 89 2399-8972

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IN2005/000132

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 5-11

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 5-11

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4, 12-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-4, 12-31
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-4, 12-31
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Reference is made to the following document:

D1: US 2003/194456

SECTION V

2. Novelty **(Article 33(2) PCT)**

The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations **(Rule 64 (1)-(3) PCT)**.
Therefore, in view of such prior art the subject-matter of **claims 1-4 and 12-31** of the present application has to be regarded as being new **(Article 33 (2) PCT)**.

3. Inventive Step **(Article 33 (3) PCT)**

The **closest prior art** to evaluate the inventiveness of the present application is document **D1**, which discloses a process for obtaining an extract from the leaves and/or stems of the *Argemone mexicana* plant and fractionation of the extract with n-butanol and methanol to give the respective n-butanol-soluble, methanol-soluble and methanol-insoluble fractions.

The present inventors have found that the methanol-insoluble fraction is constituted of arabinogalactan proteins (AGPs) in mixture with other components and provide a selective method for the isolation of AGPs in purified form from the leaves and/or stems of the *Argemone mexicana* plant.

The selective method of the present application differs from the method of D1 in that it further comprises: a) subjecting the initial aqueous extract to cation and anion exchange chromatography for removal of the basic and acid components and b) subjecting the methanol or ethanol-insoluble fraction to gel chromatography and size exclusion chromatography to obtain purified AGP.
This has not been suggested in the prior art.

The purified AGP composition obtainable by the method of the present application exhibits vastly superior antipsoriatic activity and other useful immunological and pharmacological activities compared to the extract and fractions of the leaves and/or stems of the plant *Argemone mexicana* obtainable by the method of D1.
A pharmaceutical composition comprising said purified AGP composition provides a safe, cost-effective and well-tolerated treatment for psoriasis and other diseases.

Therefore, an inventive step can be acknowledged for the subject-matter of the present application.

4. Industrial Applicability **(Article 33(4) PCT)**

The subject-matter of present **claims 1-4 and 12-31** is susceptible of industrial applicability as defined in **Article 33 (4) PCT.**

SECTION VIII

5. The present application does not satisfy the criterion set forth in **Article 6 PCT** because the following claims are not clear.

5.1. **Claim 24** refers to a purified Arabinogalactan protein (AGP).
However, the method of the present application provides a purified Arabinogalactan protein (AGP) composition and not a purified protein.
The description refers that the purified Arabinogalactan protein (AGP) composition of the present invention is constituted of several fractions of differing molecular weights, wherein such fractions have average molecular weights as high as 115 to 150 KD and as low as 10 to 15 KD (page 17, lines 27-30). The description also indicates that the components of the AGP composition have an average molecular weight range between 10 KD to 150 KD (page 17, lines 5-6).
Therefore, claim 24 should be reworded. It should be directed to a purified Arabinogalactan protein (AGP) composition obtainable by the method of the present invention and should reflect the above passages of the description.

Moreover, **claim 24** lacks clarity because the method used in the determination of the molecular weight is not indicated.

5.2. The subject-matter of **claims 24 and 26** appears to be the same.
Therefore, one of said claims is superfluous and should be deleted.

5.3. The dependency of **claim 25** on claim 59 appears to be incorrect.
It appears that said claim should depend on claim 24.

5.4. The dependencies of **claims 29-31** on claims 24 or 25 appear to be incorrect.
It appears that said claims should depend on claims 27 or 28.

********** * **********

6. In case of filling amended claims, the applicant is requested to take account of the above comments.

The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed (**Article 34 (2)(b) PCT** and **Rule 70.2 (c) PCT**).

In order to facilitate the examination of the conformity of the amended application with the requirements of **Article 34 (2)(b) PCT**, the Applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* LUP-PSOR-II

Box No. I TITLE OF INVENTION

A PURIFIED ARABINOGALACTAN-PROTEIN (AGP) COMPOSITION

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LUPIN LIMITED
159, CST Road
Kalina, Santacruz (East)
Mumbai-400 098, Maharashtra
India

Telephone No. +91 11 79 2686 8100

Facsimile No. +91 11 79 2686 2362

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ARORA, Sudershan Kumar
LUPIN LIMITED
159, CST Road
Kalina, Santacruz (East)
Mumbai-400 098, Maharashtra
India

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: IN

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☐ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SUBRAMANIAM, Hariharan; NATARAJ, Guruswamy;
AHUJA, Shalina; PRITHVIRAJ, Susheela
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi-110 048, INDIA

Telephone No. +91 11 2921 5603

Facsimile No. +91 11 2922 6005

Teleprinter No.

Agent's registration No. with the Office IN/PA-93

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SRIVASTAVA, Vandita
LUPIN LIMITED
159, CST Road
Kalina, Santacruz (East)
Mumbai-400 098, Maharashtra
India

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WALUNJ, Sameer Shankar
LUPIN LIMITED
159, CST Road
Kalina, Santacruz (East)
Mumbai-400 098, Maharashtra
India

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATIONS

The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

☐ DE Germany is not designated for any kind of national protection

☐ KR Republic of Korea is not designated for any kind of national protection

☐ RU Russian Federation is not designated for any kind of national protection

(The check-boxes above may be used to exclude (irrevocably) the designations concerned in order to avoid the ceasing of the effect, under the national law, of an earlier national application from which priority is claimed. See the Notes to Box No. V as to the consequences of such national law provisions in these and certain other States.)

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 1 September 2004 (01.09.2004)	10/931814	US		
item (2)				
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☒ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

* *Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* . . . .

. . . . . .

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA / EP . . . . . .

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:	
☒ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:	1
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:	
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:	
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:	

Box No. VIII (ii) DECLARATION: ENTITLEMENT TO APPLY FOR AND BE GRANTED A PATENT

The declaration must conform to the standardized wording provided for in Section 212; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (ii). If this Box is not used, this sheet should not be included in the request.

Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51*bis*.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate:

in relation to this International Application, LUPIN LIMITED, 159, CST Road Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India is entitled to apply for and be granted a patent by virtue of the following:

1. ARORA, Sudershan Kumar, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

2. SRIVASTAVA, Vandita, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

3. WALUNJ, Sameer Shankar, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

are the inventor for the subject matter for which protection is sought by way of this international application; and is entitled as employer of said inventors:

1. ARORA, Sudershan Kumar, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

2. SRIVASTAVA, Vandita, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

3. WALUNJ, Sameer Shankar, LUPIN LIMITED, 159, CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra, India;

this declaration is being made for the purposes of all designations except United States of America

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (ii)".

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:

(a) in paper form, the following number of sheets:

request (including declaration sheets)	:	5
description (excluding sequence listing and/or tables related thereto)	:	45
claims	:	5
abstract	:	1
drawings	:	12
Sub-total number of sheets	:	68
sequence listing	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	68

(b) ☐ only in computer readable form (Section 801(a)(i))
- (i) ☐ sequence listing
- (ii) ☐ tables related thereto

(c) ☐ also in computer readable form (Section 801(a)(ii))
- (i) ☐ sequence listing
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listing: .....
- ☐ tables related thereto: .....

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is accompanied by the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*:

	Number of items
1. ☒ fee calculation sheet	: 1
2. ☐ original separate power of attorney	:
3. ☐ original general power of attorney	:
4. ☐ copy of general power of attorney; reference number, if any:	:
5. ☐ statement explaining lack of signature	:
6. ☐ priority document(s) identified in Box No. VI as item(s):	:
7. ☐ translation of international application into *(language)*:	:
8. ☐ separate indications concerning deposited microorganism or other biological material	:
9. ☐ sequence listing in computer readable form *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:
10. ☐ tables in computer readable form related to sequence listing *(indicate type and number of carriers)*	
(i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	:
(ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	:
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:
11. ☐ other *(specify)*:	:

Figure of the drawings which should accompany the abstract:	Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Hariharan SUBRAMANIAM
Patent Attorney & Patent Agent for the Applicant
(Regn. No. IN PA/83)

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: LUP-PSOR-II

Applicant: LUPIN LIMITED et al

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . INR 8000.00 [T]

2. SEARCH FEE . . . . . USD 2075.00 [S]

International search to be carried out by EP

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FILING FEE

Where items (b) and/or (c) of Box No. IX apply, enter Sub-total number of sheets
Where items (b) and (c) of Box No. IX do not apply, enter Total number of sheets } ____

[i1] first 30 sheets . . . . . USD 1211.00 [i1]

[i2] 38 (number of sheets in excess of 30) x 13 (fee per sheet) = USD 494.00 [i2]

[i3] additional component (only if sequence listing and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

400 x ____ (fee per sheet) = ____ [i3]

Add amounts entered at i1, i2 and i3 and enter total at I . . . . USD 1705.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international filing fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the international filing fee.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . [P]

5. TOTAL FEES PAYABLE . . . . . INR 8000 + USD 3780 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

MODE OF PAYMENT

☐ authorization to charge deposit account (see below) ☐ postal money order ☐ cash ☐ coupons

☐ cheque ☐ bank draft ☐ revenue stamps ☐ other *(specify)*:

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

Receiving Office: RO/ ____

Deposit Account No.: ____

Date: ____

Name: ____

Signature: ____

☐ Authorization to charge the total fees indicated above.

☐ *(This check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

☐ Authorization to charge the fee for priority document.

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PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates,
E-556, Greater Kailash-II,
NEW DELHI- 110 048

PCT

NOTIFICATION CONCERNING PAYMENT OF PRESCRIBED FEES

(PCT Rules 14, 15 and 16 and Administrative Instructions, Sections 102*bis*(c), 304, 323(b), 707(b) and 803)

Date of mailing *(day/month/year)* 12.05.2005	
Applicant's or agent's file reference LUP-PSOR-II	PAYMENT DUE see item 3 for time limits

International application No.	International filing date/Date of receipt *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN05/00132	26 APR 2005 (26.04.05)	01 SEP 2004 (01.09.04)

Applicant LUPIN LIMITED, et al

1. The applicant is hereby notified that this receiving Office has received:

☐ the payment of all the prescribed fees, and ☐ an overpayment, which will be refunded in due course.

☒ no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balance due, as summarized under item 2, within the time limit(s) indicated under item 3.

2. Fees and payment calculation:

INR 8000 + USD 3780	–	INR 8000+ NIL	=	NIL + USD 3780
Total fees payable		Amount paid		Balance

☐ The details of the calculation are given in the Annex.

3. Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):

☒ within ONE MONTH from the date of receipt of the international application (for the transmittal fee (if any), the search fee and the international filing fee). The amount payable for each fee is the amount applicable on the date of receipt of the international application.

☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact that the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.

4. Additional observations *(if necessary)*:

☒ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed) (Rule 23.1(a) and (b)).

Name and mailing address of the receiving Office	Authorized officer
GOVT OF INDIA, PATENT OFFICE, TODI ESTATES, SUN MILL COMPOUND, LOWER PAREL (W), MUMBAI- 400 013, MAHARASHTRA,INDIA. Facsimile No. (91) (22) 2495 0622	R. BHATTACHARYA Telephone No. (91) (22) 2492 5092, 2492 4058

Form PCT/RO/102 (January 2004)

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ANNEX TO FORM PCT/RO/102
CALCULATION OF THE PRESCRIBED FEES

International application No.
PCT/IN05/00132

T Transmittal Fee

Prescribed amount: . . . . . INR 8000
Amount paid: . . . . . – INR 8000
Balance: . . . . . = NIL

S Search Fee

Prescribed amount: . . . . . USD 2075
Amount paid: . . . . . –
Balance: . . . . . =

International Filing Fee

Fixed amount for first 30 sheets: . . . . . USD 1211

38 (Number of sheets in excess of 30) x USD 13 (Fee per sheet) = USD 494

Additional component: . . . 400 x _____ (Fee per sheet) = _____

Reduction where the international application is filed *(See PCT Applicant's Guide, Volume I, General Part, for details on the availability of this reduction)*:

using the PCT-EASY software: . . . . . _____

or

in electronic form where the text of the description, claims and abstract is not in character coded format: . . . . . _____

or

in electronic form where the text of the description, claims and abstract is in character coded format: . . . . . _____

Sub-total: . . . . . USD 1705

Prescribed total amount *(The amount to be entered at I is the sub-total entered at (i1+i2+i3-r), except where the applicant is (or all applicants are) entitled to a reduction of 75%, in which case the amount to be entered at I is 25% of the sub-total (i1+i2+i3-r); certain applicants from certain States are entitled to a reduction of 75% of the international filing fee; see Notes to the Fee Calculation Sheet as annexed to the*

[redacted] USD 1705
[redacted] NIL
[redacted] USD 1705

[redacted]

[redacted] –
[redacted] –
[redacted] –

Form PCT/RO/102 (Annex) (January 2004)

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates,
E-556, Greater Kailash-II,
NEW DELHI- 110 048

PCT

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

	Date of mailing *(day/month/year)* 12.05.2005
Applicant's or agent's file reference LUP-PSOR-II	REPLY DUE within ONE months/days from the above date of mailing
International application No. PCT/IN05/00132	International filing date *(day/month/year)* 26 APR 2005 (26.04.05)
Applicant LUPIN LIMITED, et al	

1. ☒ The applicant is hereby invited, within the time limit indicated above, to correct, in the international application as filed, the defects specified on the attached:
   - ☒ Annex A
   - ☐ Annex B1 *(text matter of the international application as filed)*
   - ☐ Annex C1 *(drawings of the international application as filed)*

2. ☐ The applicant is hereby invited, within the time limit indicated above, to correct, in the translation of the international application furnished under Rule 12.3 or 12.4, the defects specified on the attached:
   - ☐ Annex A
   - ☐ Annex B2 *(text matter of the translation of the international application)*
   - ☐ Annex C2 *(drawings of the translation of the international application)*

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachments has been sent to the International Bureau
☒ and the International Searching Authority

Name and mailing address of the receiving Office PATENT OFFICE BRANCH, TODI ESTATES, 3RD FLOOR, SUN MILL COMPOUND, LOWER PAREL(WEST), MUMBAI-400 013, MAHARASHTRA, INDIA Facsimile No. (91)(22) 2492 4058, 2492 5092	Authorized officer R. BHATTACHARYA Telephone No. (91) (22) 2495 0622

Form PCT/RO/106 (January 2004)

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ANNEX A TO FORM PCT/RO/106

International application No.
PCT/IN05/00132

The receiving Office has found the following defects in the international application as filed:

1. As to signature of the international application (Rules 4.15, 26.2*bis*(a) and 90.4), the request:
   - a. ☐ is not signed* by the applicant or, if there is more than one applicant, by at least one of them
   - b. ☐ is not accompanied by the statement referred to in the check list in Box No. IX of the request explaining the lack of the signature of an applicant for the designation of the United States of America
   - c. ☒ is signed by what appears to be an agent/common representative but:
     - ☒ the international application is not accompanied by a power of attorney appointing him
     - ☐ the power of attorney accompanying the international application is not signed by all the applicants
   - d. ☐ other *(specify)*:

* Although Rule 4.15 requires that all applicants must sign the request (e.g. including all inventors/applicants for the designation of the United States of America), for the purposes of Article 14(1)(a)(i), if there is more than one applicant, it shall be sufficient that the request be signed by one of them (Rule 26.2*bis*(a)).

However, the applicant's attention is drawn to the fact that the national law applied by each designated Office may require, in connection with the processing of the international application in the national phase, that the applicant furnish the confirmation of the international application by the signature of any applicant for the designated State who has not signed the request (Rule 51*bis*.1(a)(vi)).

2. As to indications concerning the applicant* who is entitled, according to Rule 19.1, to file the international application with the receiving Office, the request (Rules 4.4, 4.5 and 26.2*bis*(b)):
   - a. ☐ does not properly indicate the applicant's name *(specify)*:
   - b. ☐ does not indicate the applicant's address
   - c. ☐ does not properly indicate the applicant's address *(specify)*:
   - d. ☐ does not indicate the applicant's nationality
   - e. ☐ does not indicate the applicant's residence

☐ Further observations about indications concerning other applicants (if applicable):

* Although Rules 4.4 and 4.5 require indications concerning the applicant, or if there are several applicants, of each of them, for the purposes of Article 14(1)(a)(ii), if there is more than one applicant, it shall be sufficient that the indications required under Rule 4.5(a)(ii) and (iii) be provided in respect of one of them who is entitled according to Rule 19.1 to file the international application with the receiving Office (Rule 26.2*bis*(b)).

However, the applicant's attention is drawn to the fact that the national law applied by each designated Office may require, in connection with the processing of the international application in the national phase, that the applicant furnish any missing indication required under Rule 4.5(a)(ii) and (iii) in respect of any applicant for the designated State (Rule 51*bis*.1(a)(vii)).

3. As to the language of certain elements of the international application, other than the description and claims (Rules 12.1(c) and 26.3*ter*(a) and (c)):
   - a. ☐ the request is not in a language of publication accepted by this receiving Office; (the) language(s) accepted by this receiving Office is/are:
   - b. ☐ the text matter of the drawings is not in the language in which the international application is to be published, which is:
   - c. ☐ the abstract is not in the language in which the international application is to be published, which is:

4. The title of the invention:
   - a. ☐ is not indicated in Box No. I of the request (Rule 4.1(a))
   - b. ☒ is not indicated at the top of the first sheet of the description (Rule 5.1(a))
   - c. ☐ as appearing in Box No. I of the request is not identical with the title heading the description (Rule 5.1(a))

5. As to the abstract (Rules 8 and 26.1(b)):
   - ☐ the international application does not contain an abstract

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
India

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
11 October 2005 (11.10.2005)	
Applicant's or agent's file reference	**International application No.**
LUP-PSOR-II	PCT/IN2005/000132

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

LUPIN LIMITED (for all designated States except US)
ARORA, Sudershan, Kumar et al (for US)

International filing date	:	26 April 2005 (26.04.2005)
Priority date(s) claimed	:	01 September 2004 (01.09.2004)
Date of receipt of the record copy by the International Bureau	:	12 September 2005 (12.09.2005)
List of designated Offices	:	

AP :BW,GH,GM,KE,LS,MW,MZ,NA,SD,SL,SZ,TZ,UG,ZM,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,HU,IE,IS,IT,LT,LU,MC,NL,PL,PT,RO,SE,SI,SK,TR
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG
National :AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BW,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,EG,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KM,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NA,NI,NO,NZ,OM,PG,PH,PL,PT,RO,RU,SC,SD,SE,SG,SK,SL,SM,SY,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VC,VN,YU,ZA,ZM,ZW

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.87.40

Authorized officer:
Tewfik BENYAHIA

Telephone No. (41-22) 338 8459

Continuation of Form PCT/IB/301

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year) 11 October 2005 (11.10.2005)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference LUP-PSOR-II	International application No. PCT/IN2005/000132

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- time limits for entry into the national phase - see updated important information (as of April 2002)
- requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated on the cover sheet of this Notification by paying national fees and furnishing translations, as prescribed by Articles 22 and 39 and the applicable national laws. In addition, the applicant may also have to comply with other special requirements applicable in certain Offices. It is the applicant's responsibility to ensure the necessary steps to enter the national phase are taken in a timely fashion. Most Offices do not issue reminders to applicants in connection with the entry into the national phase.

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 30 MONTHS from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date (see Article 39(1)), but also in respect of any designated Office, in the absence of filing of such demand, where Article22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the PCT Gazette("Section IV" part published on a weekly basis), to the PCT Newsletter (on a monthly basis) and to the relevant National Chapters in Volume II of the PCT Applicant's Guide (the paper version of which is updated usually twice a year and the Internet version of which is updated usually on a weekly basis). Finally, a cumulative table of all applicable time limits for entering the national phase is available from WIPO's Internet site, via links from various pages the site including those of the Gazette, Newsletter and Guide, at http://www.wipo.int/pct/en/index.html.

Information about the requirements for filing a demand for international preliminary examination is set out in the PCT Applicant's Guide, Volume I/A, Chapter IX. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within the time limit which is reasonable under the circumstances (Rule 17.1(a)).

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit (and all other PCT time limits) is the filing date of the earliest application whose priority is claimed (Article 2(xi)(b)).

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
INDE

Date of mailing (day/month/year) 07 March 2006 (07.03.2006)	
Applicant's or agent's file reference LUP-PSOR-II	IMPORTANT NOTIFICATION
International application No. PCT/IN2005/000132	International filing date (day/month/year) 26 April 2005 (26.04.2005)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 01 September 2004 (01.09.2004)
Applicant LUPIN LIMITED et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)* An asterisk (*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
01 September 2004 (01.09.2004)	10/931,814	US	21 February 2006 (21.02.2006)

The International Bureau of WIPO
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1211 Geneva 20, Switzerland

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WO 2006/025088
PCT/IN2005/000132

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

FIRST NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION (TO DESIGNATED OFFICES WHICH DO NOT APPLY THE 30 MONTH TIME LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
INDE

Date of mailing (*day/month/year*)	
06 April 2006 (06.04.2006)	
Applicant's or agent's file reference	IMPORTANT NOTICE
LUP-PSOR-II	

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/IN2005/000132	26 April 2005 (26.04.2005)	01 September 2004 (01.09.2004)

Applicant

LUPIN LIMITED et al

1. **ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), does apply, please see Form PCT/IB/308(Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, does not apply, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93*bis*.1. The International Bureau has effected that communication on the date indicated below:
09 March 2006 (09.03.2006)

   CH

   In accordance with Rule 47.1(c-*bis*)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, does not apply, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93*bis*.1:

   LU, SE, TZ, UG, ZM

   In accordance with Rule 47.1(c-*bis*)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. **TIME LIMITS for entry into the national phase**

   For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of 19 months from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be **20 MONTHS** from the priority date.

   In practice, time limits other than the 20-month time limit will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For regular updates on the applicable time limits (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at: http://www.wipo.int/pct/en/index.html

   It is the applicant's sole responsibility to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Dorothée Mülhausen
Facsimile No.+41 22 740 14 35	Facsimile No.+41 22 338 87 40

Form PCT/IB/308(First Notice) (January 2004)

WO 2006/025068
PCT/IN2005/000132

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
INDE

Date of mailing *(day/month/year)* 09 March 2006 (09.03.2006)		
Applicant's or agent's file reference LUP-PSOR-II		IMPORTANT NOTICE
International application No. PCT/IN2005/000132	International filing date *(day/month/year)* 26 April 2005 (26.04.2005)	Priority date *(day/month/year)* 01 September 2004 (01.09.2004)
Applicant LUPIN LIMITED et al		

The International Bureau transmits herewith the following documents:

☑ copy of the international application as published by the International Bureau on 09 March 2006 (09.03.2006) under No. WO 2006/025068

☐ copy of international application as republished by the International Bureau on under No. WO
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) (*as the case may be*) on the front page of the attached document.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Dorothée Mülhausen
Facsimile No.+41 22 740 14 35	Facsimile No.+41 22 338 87 40

Form PCT/IB/311 (January 2004)

WO 2006/025068
PCT/IN2005/000132

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
INDE

Date of mailing (*day/month/year*)	
20 April 2006 (20.04.2006)	
Applicant's or agent's file reference LUP-PSOR-II	IMPORTANT NOTICE

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/IN2005/000132	26 April 2005 (26.04.2005)	01 September 2004 (01.09.2004)

Applicant
LUPIN LIMITED et al

The International Bureau transmits herewith the following documents:

☐ copy of the international application as published by the International Bureau on under No. WO

☑ copy of international application as republished by the International Bureau on 20 April 2006 (20.04.2006) under No. WO 2006/025068
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) (*as the case may be*) on the front page of the attached document.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Dorothée Mülhausen
Facsimile No. +41 22 740 14 35	Facsimile No. +41 22 338 87 40

Form PCT/IB/311 (January 2004)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
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(43) International Publication Date
9 March 2006 (09.03.2006)

PCT

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WO 2006/025068 A1

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(21) International Application Number: PCT/IN2005/000132

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/931,814 1 September 2004 (01.09.2004) US

(71) Applicant (*for all designated States except US*): LUPIN LIMITED [IN/IN]; 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharashtra (IN).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): ARORA, Sudershan, Kumar [US/IN]; Lupin Limited, 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharashtra (IN). SRIVASTAVA, Vandita [IN/IN]; Lupin Limited, 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharashtra (IN). WALUNJ, Sameer, Shankar [IN/IN]; Lupin Limited, 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharashtra (IN).

(74) Agents: SUBRAMANIAM, Hariharan et al.; Subramaniam, Nataraj & Associates, E-556, Greater Kailash-II, New Delhi 110 048 (IN).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report*

(48) Date of publication of this corrected version:
20 April 2006

(15) Information about Correction:
see PCT Gazette No. 16/2006 of 20 April 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A PURIFIED ARABINOGALACTAN-PROTEIN (AGP) COMPOSITION

(57) Abstract: A purified Arabinogalactan-Protein (AGP) composition isolated through a selective method from the leaves and/or stems of *Argemone mexicana* plant is described. Also described is a purified Arabinogalactan-Protein (AGP) composition isolated from the leaves and/or stems of *Argemone mexicana* plant, which has one or more of the following effects: immunosuppression, lymphoproliferation inhibition, cytokine modulation such as IL-2 inhibition, IFN-γ inhibition, or IL-10 induction; keratinocyte proliferation inhibition, keratolytic activity and inhibitory activity in Mouse Ear Swelling test (MEST).

WO 2006/025068 A1

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Article 31(7) and Rule 61.3)

To:

SUBRAMANIAM, Hariharan
Subramaniam, Nataraj & Associates
E-556, Greater Kailash-II
New Delhi 110 048
INDE

Date of mailing *(day/month/year)* 18 May 2006 (18.05.2006)	
Applicant's or agent's file reference LUP-PSOR-II	IMPORTANT INFORMATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN2005/000132	26 April 2005 (26.04.2005)	01 September 2004 (01.09.2004)

Applicant

LUPIN LIMITED et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR
   National: BG, CA, CN, CZ, DE, JP, KM, KP, KR, MN, NO, PL, RO, RU, SK, SM, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   AP: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW
   EA: AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
   OA: BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
   National: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BR, BW, BY, BZ, CH, CO, CR, CU, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MW, MX, MZ, NA, NI, NZ, OM, PG, PH, PT, SC, SD, SE, SG, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

3. Since the election(s) was (were) made before the expiration of 19 months from the priority date, the applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary report on patentability (Chapter II of the Patent Cooperation Treaty) (Article 36(3)(b) and Rule 74.1).

Some Offices have fixed time limits expiring later than the above-mentioned time limit. See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters, the *PCT Newsletter* and the WIPO Internet site, updated regularly.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.+41 22 740 14 35

Authorized officer

Dorothée Mülhausen

Facsimile No.+41 22 338 87 40

Industria y Comercio
SUPERINTENDENCIA

REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [X] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [X]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- Descripción de la invención [X]
- Dibujos de ser estos pertinentes [X]
- Comprobante de pago de las tasas establecidas [X]

COMPLETA [ ] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Claudia M Neira
Firma Responsable

Fecha_____

Carrera 13 No. 27-00 Piso 5, 7 y 10
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E-mail: info@sic.gov.co
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Bogotá, D.C., Colombia

224

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

FOLIO 296

2020
Bogotá D.C.,

Doctor(a)
RODRIGUEZ DILIA MARIA
Apoderado(a) y/o Representante de
LUPIN LIMITED

REFERENCIA	Radicación No.	07 28598
	Solicitud internacional	PCT/IN/2005/000132
	Fecha inicio fase nacional	01/04/2007
	Trámite	11
	Evento	379
	Actuación	416
	Oficio No.	2982
	Folios	1

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en la Decisión 486 de la Comisión de la Comunidad Andina, el Tratado de Cooperación en Materia de Patentes (PCT), el Reglamento y la Circular Única de la Superintendencia de Industria y Comercio, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial.

Cúmplase
Dado en Bogotá DC, 03 MAY 2007

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

adpa11