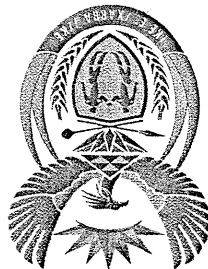


REPUBLIEK VAN SUID AFRIKA



REPUBLIC OF SOUTH AFRICA

PATENTS ACT, 1978

## CERTIFICATE

In accordance with section 44 (1) of the Patents Act, No. 57 of 1978, it is hereby certified that:

SAHARAN, Pawan

Has been granted a patent in respect of an invention described and claimed in complete specification deposited at the Patent Office under the number

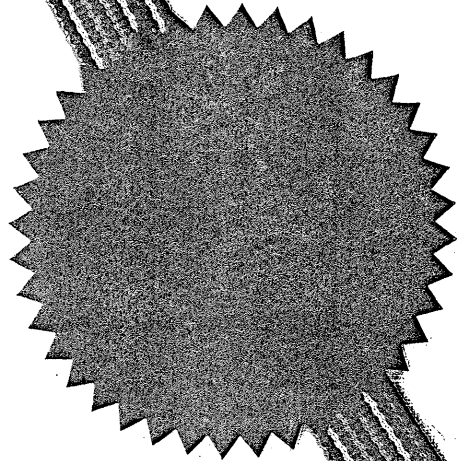
2011/04687

A copy of the complete specification is annexed, together with the relevant Form P2.

In testimony thereof, the seal of the Patent Office has been affixed at Pretoria with effect

from the 29 day of February 2012

Registrar of Patents



REPUBLIC OF SOUTH AFRICA  
 PATENTS ACT, 1978  
 REGISTER OF PATENTS

FORM P2

21	21	21	21	21	21
OFFICIAL APPLICATION NO.	LODGING DATE: PROVISIONAL	ACCEPTANCE DATE	INTERNATIONAL CLASSIFICATION	LODGING DATE: COMPLETE	GRANTED DATE
			2011/04687		10-01-2012
51	23	23	51	23	23
INTERNATIONAL APPLICATION NO.	LODGING DATE: INTERNATIONAL		A61K C07K	LODGING DATE: COMPLETE	24 Jun 2011
			PCT/IN2009/000749	LODGING DATE: INTERNATIONAL	29 Dec 2009

74	FULL NAME(S) OF APPLICANT(S)/PATENTEE(S)				
	SAHARAN, Pawan				
71	APPLICANTS SUBSTITUTED				
	DATE REGISTERED				
71	ASSIGNEES				
	DATE REGISTERED				
71	FULL NAME(S) OF INVENTOR(S)				
	SAHARAN, Pawan				

74	PRIORITY CLAIMED				
	COUNTRY	NUMBER	DATE	NB - Use International abbreviation for country (See Schedule 4)	
	IN	1353/MUM/2008	27 Dec 2008		

54	TITLE OF INVENTION				
	MAMMALIAN COLOSTRUM DERIVED NANOPETIDES FOR BROADSPECTRUM VIRAL AND RECURRENT INFECTIONS WITH A METHOD OF ISOLATION THEREOF				
	ADDRESS OF APPLICANT(S)/PATENTEE(S)				
	A 2101-04, MANSAROVAR NEELKANTH HEIGHTS, POKHRAN ROAD, NO. 1, THANE (W) 400 601 MAHARASHTRA, INDIA				
61	PATENT OF ADDITION NO.				
	DATE OF ANY CHANGE				
	FRESH APPLICATION BASED ON				
	DATE OF ANY CHANGE				

74	ADDRESS FOR SERVICE				
	HAHN & HAHN INC., HAHN FORUM, 222 RICHARD STREET, HATFIELD, 0083 PRETORIA				
	REF				
	PCT/IN09/749				



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
15 July 2010 (15.07.2010)

(10) International Publication Number  
WO 2010/079511 A2



(51) International Patent Classification:  
A61K 38/10 (2006.01)  
A61K 38/04 (2006.01)  
C07K 7/04 (2006.01)

(21) International Application Number:  
PCT/IN2009/000749

(22) International Filing Date:  
29 December 2009 (29.12.2009)

(25) Filing Language: English  
(26) Publication Language: English

(30) Priority Data:  
1553/MUM/2008 27 December 2008 (27.12.2008) IN

(72) Inventor; and  
(71) Applicant : SAHARAN, Pawan [IN/IN]; A 2101-04,  
Mansarovar Neelkamth Heights, Pokhran Road, No. 1,  
Thane (W) 400 601, Maharashtra (IN).

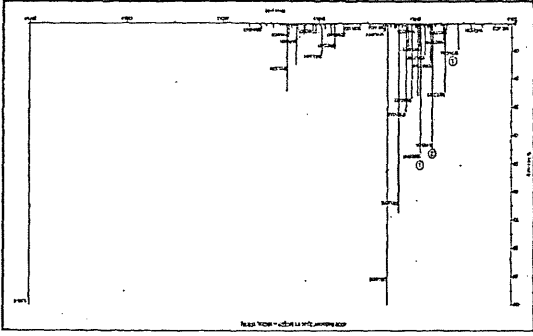
(74) Agents: SAURASTRI, Manish et al.; Krishna & Sauras-  
tri Associates, 74/F, Venus World Sea Face, Mumbai 400  
018, Maharashtra (IN).  
Published: without international search report and to be republished  
upon receipt of that report (Rule 48.2(g))  
— kind of national protection available: AE, AG, AT, AM,  
AO, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,  
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AT, AM,  
AO, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,  
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,  
— with sequence listing part of description (Rule 5.2(a))

(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, HR, HU, IE, IS, IT, LT, LU, LV,  
MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM,  
TR), OAPI (BF, BJ, CF, CG, CI, CM, GN, GQ, GW,  
ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:  
— of inventorship (Rule 4.17(iv))  
— Published:

(54) Title: MAMMALIAN COLOSTRUM DERIVED NANOPETIDES FOR BROADSPECTRUM VIRAL AND RECUR-  
RENT INFECTIONS WITH A METHOD OF ISOLATION THEREOF



(57) Abstract: The present invention relates to nanopetides isolated from mammalian colostrum with vaccine like antiviral and immunomodulator activity via building body's own immune system and attachment inhibition on the cell surface receptors.

WO 2010/079511 A2



FORM CONTINUATION SHEET

Date of filing	
Date of publication	
Date of withdrawal	
Date of cancellation	
Date of restoration	
Date of re-examination	
Date of appeal	
Date of opposition	
Date of revocation	
Date of reinstatement	
Date of other proceedings	

TITLE OF THE INVENTION

MAMMALIAN COLOSTRUM DERIVED NANOPETIDES FOR BROADSPECTRUM VIRAL AND  
RECURRENT INFECTIONS WITH A METHOD OF ISOLATION THEREOF

FIELD OF THE INVENTION

The present invention relates to nanopetides isolated from mammalian colostrum with  
vaccine like antiviral and immunomodulator activity via building body's own immune  
system and attachment inhibition on the cell surface receptors.

BACKGROUND OF THE INVENTION

Colostrum is the pre-milk substance produced from the mother's breasts of all  
mammals during the first 24 hrs of lactation typically first 3 milks. Colostrum has been  
known as an immune booster since time immemorial. Colostrum triggers at least 50  
processes in the newborn, including transferring all immune factors and the entire  
memory from mother's own immune system. Bovine colostrum is upto 40 times higher  
than human colostrums in immune factors including nanoinformational peptides,  
Proline Rich Polypeptides, immunoglobulins, cytokines, interferon, lactoferrin and  
transfer factor. They are produced by T-lymphocytes and can transfer the ability to  
recognize a pathogen to naive cells. However, no one till date has been able to isolate  
active ingredients especially nano informational low molecular weight nanopetides and  
formulate a product that has the same effect that mother's first 3 milk, after the birth of  
child.

"Colostrum stimulates the lymphoid tissue providing benefits in aged or immuno-  
deficient people"....Drs. Bocci, Bremen, Corradeschi, Luzzi and Paulesu; Journal Biology.

"Researchers reported that colostrum stimulates maturation of B Lymphocytes (type of white blood cell) and primes them for production of antibodies, enhances growth and differentiation of white blood cells. Similar activity in cow and human colostrum can also activate Macrophages" ...Dr. M. Julius, McGill University, Montreal: Science News.

"Bovine colostrum contains high levels of growth factors that promote normal cell growth and DNA synthesis" ...Drs. Oda, Shinichi, et. al.;

10 Comparative Biochemical Physiology. "Drs. suggest that an important role for growth factors is in promoting wound healing. Accelerated healing is possible for treatment with trauma and surgical wounds" ...Drs. Bhora, et. al.; Journal. Surg. Res.

15 US 20070212367 – This patent application discloses an immunologically active RRP isolated from mammalian colostrum fluids for treatment of viral and non-viral diseases, a method and a system for processing mammalian colostrum fluids and a pharmaceutical formulation.

20 Colostrum as such contains hundreds of small peptides which serve numerous purposes. Their segregation and isolation will facilitate gathering of further information with regard to their individual function and help formulate specific and targeted therapies for numerous diseases that are cured by colostrum. Studies have documented the presence of number of bioactive peptides but no mention has been made of the use of these peptides fragments, their specific sequence or information regarding their isolation. The peptides are extremely sensitive to temperature, pH, stress and shear factors which posse several difficulties in their isolation and preserving their biological activity. and method of collection of colostrum so as to deliver it to the required patient by maintaining its full biological activity.

Summary of the Invention

The present invention addresses these shortcomings by providing isolated nano-peptides from colostrum, their method of isolation and therapeutic uses of the isolated nano-peptide fragments.

One embodiment of the present invention provides for a formulation comprising peptides isolated from mammalian colostrum having sequences as recited in SEQ ID 1-8 hereinafter referred to as peptides of Radha 108 series, wherein the peptides function to modulate cell immunity and provide attachment inhibition for foreign antigen / viruses on cell surface receptors along with crossing BBB (blood brain barrier) and treating host of diseases in the brain

Colostrum is the pre-milk substance produced from the mother's breasts of all mammals during the first 24 hrs of lactation typically first 3 milks. In one embodiment, colostrum used in the present invention is of bovine origin. Bovine colostrum has 40 times higher immune factors than human colostrum and has the ability to cure a number of viral, immune and auto-immune disorders. In another embodiment, there is provided a method of treatment of immune related disorders including autoimmune disorders the method comprising of administering a patient suffering from such disorders a therapeutically effective amount of formulation comprising the peptides of SEQ ID 108.

In yet another embodiment, there is provided a method of treatment of Acquired Immune Deficiency Syndrome or AIDS the method comprising administering a patient suffering from AIDS, a therapeutically effective amount of formulation comprising the peptides of SEQ ID 1-8.

The formulation can be provided in a liquid, powder gel and any other pharmaceutical delivery forms. It can be administered orally, intravenously or by means of dermal patch for adsorption through skin.

The present invention is illustrated with the help of accompanying drawings and detailed description and examples given below. The drawings and example are for explanation and clarity purpose and do not in any way limit the scope of the invention being defined by the appended claims and equivalents thereof. The foregoing aspects and advantages of the present invention will become more clear and appreciated by the detailed description and examples.

Brief Description of Figures

Fig 1: Resolution of bovine colostrum sample used in the present invention through MS Spectra to give various protein fragments of different molecular weights.

A BRIEF DESCRIPTION OF THE INVENTION

The present invention relates to peptides isolated from colostrum, their innovative methods of collection, transport, storage, isolation and therapeutic uses of the same.

One embodiment of the present invention relates to peptides isolated from mammalian colostrum. The mammal can be bovine for example cow, goat, buffalo or any other suitable mammal. In one preferred embodiment of the present invention, the isolated peptides are obtained from lactating cows and buffalos in particular. The peptides are short chain amino acid sequences which are fragile sensitive to temperature, pH and shear stress and difficult to isolate. These peptides are in a segregated form. They are not binded by any other molecule. The isolated peptides comprise of the group of SEQ ID (1-8) and will be hereinafter referred to as Radha 108. Peptides of Radha 108 and do not coagulate being low in molecular weight. They are isolated from fat free whey



obtained from the colostrum. The peptides of Radha 108 have a molecular weight in the range of 826 to 2990 kDa.

According to one embodiment of the present invention there is provided a method for collection and processing colostrum and isolating small amino acid sequences

comprising of SEQ ID (1-8) the method comprising the steps of, separating fat, cheese and colostrum whey, passing the whey through a series of filters to remove suspended solids and reduce microbial load followed by ultra and nano filters to obtain a protein fraction having a molecular weight of less than 1000kDa, specifically below 350kDa wherein the filtration is done at a low pressure, minimal shear and temperature to

preserve the Radha 108 nano peptides. Even the pumping means used to pump the liquid through the system consist of pumps with low rpm. In one embodiment, the nano and ultra filters are designed to operate at a low shear and pressure of less than 5 kg/cm<sup>2</sup>. In another preferred embodiment, the filters are designed to operate in the range of 0.5kg/cm<sup>2</sup> to 4kg/cm<sup>2</sup>. In yet another preferred embodiment, the filters are designed to operate at a pressure range between 0.5kg/cm<sup>2</sup> and 2.0 kg/cm<sup>2</sup>. Nano and ultra filtration of proteins at such low pressures is virtually unheard off due to

practical difficulties in obtaining effective amounts of protein within acceptable time limits. The present invention thus overcomes the problem of prior art with regard to isolation and separation of small peptide molecules.

Colostrum is collected after delivery from mammal, and cooled to a temperature in the range of -20 deg C within 3hours to preserve the biological activity of Radha 108 series nano peptides. Care is taken to preserve these sensitive peptides right from the collection stage by provisions of special custom made non reactive plastic bags which help in the non reactive aseptic collection of milk from the calf. The handlers and collector of the milk are to be provided special instruction for the collection in order to avoid drastic changes to the sensitive peptides so that they don't lose their activity. The colostrum is then gradually thawed at room temperature over night to bring the

temperature to 4 to 5 deg C, and pumped to a cream separator for removal of fats at 4000 RPM at 45 deg C. It is then pasteurized by increasing the temperature to 72 deg C for 15 sec followed by cooling to 48 deg C. The colostrum is then received into cheese vat and treated with enzyme Rennet for 45-60 min to form curd and whey. The whey is cooled and passed through a series of filters upto molecular exclusion nano and ultra-filtration at low pressure and temperature for separation to peptides having a molecular weight of less than 10000kDa. After the process care is taken to adjust the pH and keep it constant in the range of pH 4 to pH 6 in order to avoid degradation and loss of biological activity of the peptides of Radha 108. The protein fraction containing peptides less than 10000 kDa

5

In another embodiment of the present invention there is provided a method of treating Acquired Immune Deficiency Syndrome or AIDS, the method comprising administering a patient suffering from AIDS, a therapeutically effective amount of a formulation comprising peptides of SEQ ID 1-8.

10

The peptides of the present invention block receptors GP120, 160, 180, 41 and other cell receptor sites through which foreign antigen/virus enters the immune and other cell types. Topographically, these peptides are similar to virus size and when they dock on these receptor sites mimicking the virus / antigen, thereby limiting the entry of foreign infectious agents though competitive attachment inhibition. The product also has a vaccine like effect via providing memory to B Cells at stem cell level and preventing future outbreak of the disease.

20

In another embodiment of the present invention there is provided a method of treating swine flu in mammals, the method comprising administering a patient suffering from swine flu, a therapeutically effective amount of a formulation comprising peptides of Radha 108.

25

In another embodiment of the present invention there is provided a method of treating immune disorders including autoimmune disorders comprising administering a patient suffering from such disorders a pharmaceutically effective dosage / amount of formulation comprising peptides of Radha 108.

The immuno-modulatory action of peptides of Radha 108 takes place by stimulating the

maturation of immature thymocytes into either helper or suppressor T cells, depending on the need of the body. Helper T cells present antigens (such as viral protein) to B

lymphocytes, which produce antibodies to that antigen. Helper T cells also help produce memory T cells, which retain the memory of an antigen in order to expedite the

production of antibodies in the event the antigen is re-encountered in the future creating vaccine like memory for the first time in history of use of colostrum. Suppressor

T cells, on the other hand, deactivate other lymphocytes after an infection has been cleared to avoid damage to healthy tissues. Peptides of the present invention also

promote the growth and differentiation of B cells in the response to an infection and the differentiation and maturation of macrophages and monocytes. The activity of Natural

Killer cells from Thymus gland via Radha 108 series of peptide that crosses BBB(Blood Brain Barrier), cytotoxic cells of the innate immune system, was increased up to 5 times

by the peptides (Radha 108) of the present invention.

The peptides of the present invention are also known to modulate the cytokine system as well. It stimulates the production of a wide range of cytokines, including the pro-

inflammatory cytokines tumor necrosis factor – alpha (TNF-alfa and gamma (INF – -inflammatory cytokines interleukin (IL1, IL2, IL6, IL10, IL11, IL12) immuno-modulators.

The peptides of the present invention are small nanopeptides ranging between 800 to 3200 daltons and hence they are able to cross the blood brain barrier, and treat viral

infections and neural disorders in brain via signaling Pituitary gland located in the brain .