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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 August 2012 (02.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/101008 Al

(51) International Patent Classification:
A61K 36/47 (2006.01)

(21) International Application Number:
PCT/EP2012/050658

(22) International Filing Date:
17 January 2012 (17.01.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

233/MUM/2011	27 January 2011 (27.01.2011)	IN
245/MUM/2011	28 January 2011 (28.01.2011)	IN
61/444,803	21 February 2011 (21.02.2011)	US
61/445,046	22 February 2011 (22.02.2011)	US
569/MUM/2011	1 March 2011 (01.03.2011)	IN
61/452,730	15 March 2011 (15.03.2011)	US

(71) Applicant *(for all designated States except US):* **GLENMARK PHARMACEUTICALS LIMITED** [IN/IN]; Glenmark House, HDO - Corporate Bldg, Wing A, B. D. Sawant Marg, Chakala, Anderi (East), Mumbai 400 099 (IN).

(72) Inventor; and
(71) Applicant *(for MW only):* **ATKINSON, Jonathan David Mark** [GB/GB]; c/o Harrison Goddard Foote, Belgrave Hall, Belgrave Street, Leeds West Yorkshire LS2 8DD (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only):* **KHAN, Mubeen Ahmad** [IN/IN]; H2-0:1, Panchdeep Society, Sector-29, Vashi, Navi Mumbai, Maharastra 400 703 (IN). **SINHA, Sukumar** [IN/IN]; Prajapati Lawns, Plot No-7, Flat No-A-701, Sector-6, Kharghar, Navi Mumbai, Maharashtra 410 210 (IN). **PARASHAR, Vikas** [IN/IN]; 6/105, Garden Estate, Ruby CHS Ltd, Glady Alwaris Rd, Thane (W), Maharashtra 400 610 (IN). **PANDEY, Vipin Kumar** [IN/IN]; Flat No. 304, Bldg. No. 55, River Wood Park, Kalyan Shil Road, Sagarli Village, Thane, Maharastra 400 612 (IN). **SHAH, Snehal** [IN/IN]; B/33, Abhilasha Bungalows, Opp. National High School, Near Sardar Park Chawkdi GIDC, Ankleshwar Dist. Bharuch, Gujarat 393 002 (IN).

(74) Agent: **HARRISON GODDARD FOOTE;** Belgrave Hall, Belgrave Street, Leeds West Yorkshire LS2 8DD (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, 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, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

WO 2012/101008 A1

(54) Title: PROCESS FOR THE PRODUCTION OF PROANTHOCYANIDIN POLYMERIC COMPOSITION

(57) Abstract: The present patent application relates to a process for the isolation and purification of a proanthocyanidin polymeric composition, the composition having high purity and being suitable for use in pharmaceutically effective formulations. In particular, the invention relates to a process for the production of Crofelemer suitable for pharmaceutical formulations and having a level of purity and concentration which enable it to be used in a therapeutically effective manner.

PROCESS FOR THE PRODUCTION OF PROANTHOCYANIDIN POLYMERIC COMPOSITION

Related applications

This application claims benefit of Indian provisional application No(s). 233/MUM/2011 filed on January 27, 2011; 245/MUM/201 1 filed on January 28, 2011; 569/MUM/2011 filed on March 1, 2011; and US provisional application No(s). 61/444,803 filed on February 21, 2011; 61/445,046 filed on February 22, 2011; 61/452,730 filed on March 15, 2011; all of which are hereby incorporated by reference in their entirety.

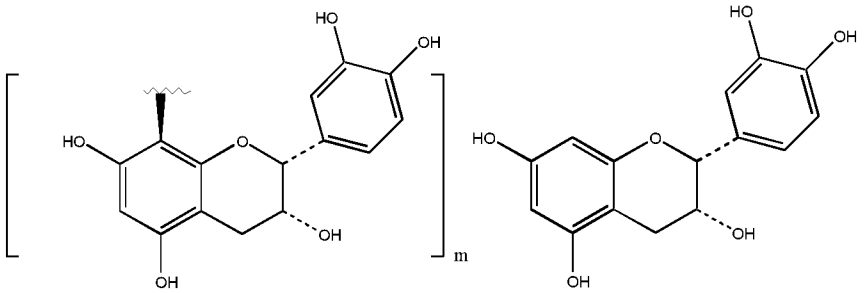
Technical Field

The present invention relates to a process for the isolation and purification of a proanthocyanidin polymeric composition, the composition having high purity and being suitable for use in pharmaceutically effective formulations. In particular, the invention relates to a process for the production of Crofelemer suitable for use in a pharmaceutical formulation and having a level of purity and concentration that enables it to be used in a therapeutically effective manner.

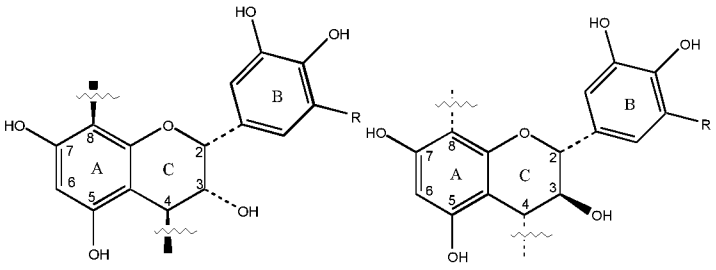
The present invention also relates to a proanthocyanidin polymeric composition, and in particular Crofelemer, obtainable from the process of this invention. In addition, the invention also provides a proanthocyanidin polymeric composition and/or proanthocyanidin polymers, and in particular Crofelemer, obtained (whether directly or indirectly) by means of the process of the invention.

Background of the Invention

Proanthocyanidin and proanthocyanidin polymers are phenolic substances found as colorless or brownish naturally occurring substances in a wide variety of many plants, particularly those with a woody habit of growth (e.g., the *Croton species* and *Calophyllum inophylum).* The general chemical structure of a polymeric proanthocyanidin consists of linear chains of 5, 7, 3', 4' tetrahydroxy or 5, 7, 3', 5' pentahydroxy flavonoid 3-ol units linked together through common C(4)-(6) and/or C(4)-C(8) bonds, as shown below.



Biosynthetic studies have indicated that proanthocyanidin polymers consist of monomer units of the type shown below, See Fletcher et al, 1977, J.C S. Perkin, 1:1628



R = H, OH R = H, OH

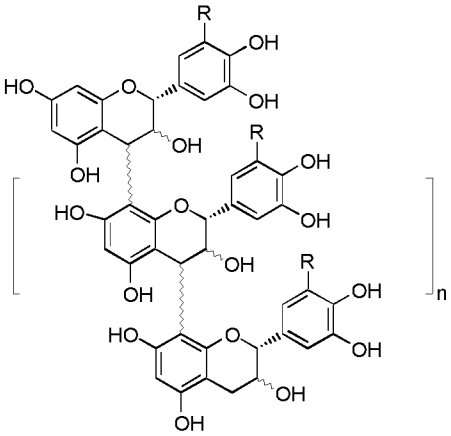
The monomer unit (generally termed "leucoanthocyanidin") of the polymer chain may be based on either of two stereochemistry of the C-ring, at the 2 and/or 4 position designated cis (called epicatechins) or trans (called catechin). Therefore, the polymer chains are based on different structural units, which create a wide variation of polymeric proanthocyanidins and a large number of possible isomers.

Proanthocyanidins have a variety of biological activities including antitumor, anti-inflammatory, anti-aging, antioxidant, antiallergy, antibacterial, and hair growth activities.

Crofelemer is a member of this proanthocyanidins class. It is a phenolic polymer isolated from the red and viscous latex of the plant species *Croton lechleri* (Euphorbiaceae). This latex, commonly referred to as "Sangre de Drago" or "Sangre de Grado" ("dragon's blood"), is one of the most common traditional herbal medicines in Latin America. As well as containing Crofelemer, "Sangre de Drado" has also been found to contain an alkaloid identified as taspine. Taspine (CAS registry number 602-07-33; 8-dimethoxy-l-[2-(dimethylamino)ethyl][l]benzopyrano[5,4,3-cde][l]benzopyran-5,10-dione) has also been shown to have various pharmaceutical properties, such as bacteriostatic properties, wound healing properties, cytotoxicity, immunosuppression

activity, acetylcholinesterase inhibition, and inhibitory effects on the activity of tumor angiogenesis.

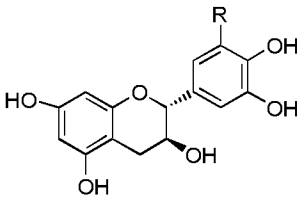
Crofelemer is a polymeric composition with CAS Registry number 148465-45-6 and is chemically represented by the following structural formula:



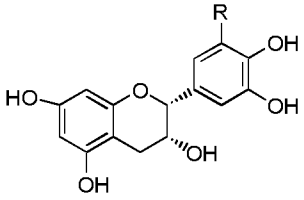
1

R= H (procyanidin) and/or R= OH (prodelphinidin)
Average n ~ 5 (7 units)

wherein, structure of monomeric units are:



2 R= H, (+)-catechin
4 R= OH, (+)-gallocatechin



3 R= H, (-)-epicatechin
5 R= OH, (-)-epigallocatechin

As a polymeric composition, Crofelemer may additionally contain one of more impurities, for example taspine or other impurities such as acetone, n-butanol and diacetone alcohol.

U.S. Patent No. 5211944 first described the isolation of an aqueous soluble proanthocyanidin polymer composition from *Croton spp.* and the use of the composition as an antiviral agent. The proanthocyanidin polymer composition was shown to have antiviral activity against a variety of viruses including, respiratory syncytial, influenza, parainfluenza and herpes viruses. More recently, Crofelemer has been found to be useful in the treatment of diarrhea and is currently being developed for the treatment of secretory diarrhea. Secretory diarrheas (also known as watery diarrheas) constitutes a major source of mortality and morbidity worldwide, particularly in developing countries

and particularly in relation to infants and young children. Secretory diarrhea is caused by a variety of pathogens including bacteria, viruses and protozoa, as well as by other non-infectious etiologies such as abnormal cell proliferation in the gastrointestinal tract, for example cancer. It is characterized by the increased secretion of aqueous fluids into the bowel resulting in the loss of fluid and electrolytes through the gastrointestinal tract. This can lead to serious dehydration.

The development of Crofelemer as a treatment for diarrhea represents significant progress in the treatment of numerous conditions in which diarrhea, and particularly secretory diarrhea, is a symptom. Clinical trials have shown that Crofelemer is active in the treatment of diarrhea in people living with HIV and AIDS and Irritable Bowel Syndrome (IBS) and in connection with acute infectious diarrhea (including cholera) and pediatric diarrhea. There is thus significant medical need for Crofelemer and consequently for efficient, cost-effective and environmentally friendly methods for preparing Crofelemer and Crofelemer compositions. Methods for isolating proanthocyanidin polymer compositions are known in the art. In particular U.S. Patent No. 521 1944 discloses in detail a method for isolating a proanthocyanidin polymer composition. U.S. Patent No. 7323195 also discloses a method for isolating a proanthocyanidin polymer composition. However, despite these disclosures the large scale production of proanthocyanidin polymers, such as Crofelemer, and their associated compositions is a difficult and challenging task in terms of cost effective production as well as with regard to waste management and yield. Therefore, there remains a need for an improved process for the isolation of a proanthocyanidin polymer composition such as a Crofelemer composition with high purity and high yield for use in pharmaceutical formulations. Surprisingly, the inventors have been able to improve the prior art methods for the production of Crofelemer, in relation to the quality of the product obtained. The method of the present invention utilizes an elevated temperature (when compared to the prior art methods) and this method produces in Crofelemer having improved purity and yield.

Object of the Invention

It is thus an object of this invention to provide a process for the production of a proanthocyanidin polymer composition such Crofelemer with improved purity, for example with reduced levels of taspine.

It is also an object of this invention provide a process for the production of a proanthocyanidin polymer composition such as Crofelemer with improved yield.

It is another object of this invention to provide a process for the production of a proanthocyanidin polymer composition such as Crofelemer which is more environmentally friendly that than any of the previous disclosed processes.

It is a further object of this invention to provide a process for the production of a proanthocyanidin polymer composition such as Crofelemer which is more efficient than any of the previous disclosed processes.

It is another object of this invention to provide a process for the production of a proanthocyanidin polymer composition such as Crofelemer which is more cost effective than any of the previous disclosed processes.

The present invention relates to process for the production of a proanthocyanidin polymer composition such as Crofelemer which addresses each of the above mentioned objects individually or in any combination thereof.

Summary of the Invention

The present invention relates to a process for the isolation and purification of a proanthocyanidin polymeric composition, the composition having high purity and being suitable for use in pharmaceutically effective formulations. In particular, the invention relates to an efficient, economical and/or environmentally friendly process for the production of Crofelemer suitable for pharmaceutical formulations and having a level of purity and concentration that enables it to be used in a therapeutically effective manner.

The present invention also relates to a proanthocyanidin polymeric composition, and in particular Crofelemer, obtainable from the process of the invention. In addition, the invention also provides a proanthocyanidin polymeric composition and/or proanthocyanidin polymers, and in particular Crofelemer obtained (whether directly or indirectly) by means of the process of the invention.

In one aspect, the present invention provides Crofelemer having a polydispersity index in the range of 0.9 to 1.2, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

In another aspect, the present invention provides Crofelemer wherein taspine is present in an amount of less than 500 ppm; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique.

In yet another aspect of the present invention, there is provided Crofelemer having an assay of greater than 85%, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique.

In yet another aspect of the present invention, there is provided Crofelemer having less than 0.15% of an impurity, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique. In one embodiment of this aspect Crofelemer has less than 0.15% of an impurity as measured at RRT 0.07.

In yet another aspect of the present invention, there is provided Crofelemer having water content in the range of 7% to 17% (wt %), obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique. In one embodiment of this aspect, the water content was analyzed by KF technique.

In yet another aspect, the present invention provides a method of producing Crofelemer, which method comprises the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique.

In yet another aspect of the present invention there is provided a process for isolating partially purified Crofelemer (as described in step (A) of the aforementioned aspects of the invention), comprising the steps of:

(a) stirring a mixture of crude plant latex or freeze-dried powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C;

(b) separating the liquid phase;

(c) optionally concentrating the liquid phase to obtain solid, liquid or concentrated syrup; and

(d) optionally adding water or water miscible solvent(s) to the solid, liquid or concentrated syrup.

In yet another aspect of the present invention, there is provided Crofelemer having a polydispersity index in the range of 0.9 to 1.2, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of the present invention, there is provided Crofelemer wherein taspine is present at an amount of less than 500 ppm, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of present invention, there is provided Crofelemer having an assay of greater than 85%, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-

column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of present invention, there is provided Crofelemer having less than 0.15% of an impurity, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1. In one embodiment of this aspect Crofelemer has less than 0.15% of an impurity as measured at RRT 0.07.

In yet another aspect of present invention, there is provided Crofelemer having water content in the range of 7-17 % (wt %), obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by a using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1. In one embodiment of this aspect, the water content was analyzed by KF technique.

In yet another aspect of present invention, there is provided Crofelemer having purity of greater than about 98%.

In yet another aspect of present invention, there is provided Crofelemer having an assay of greater than about 85%.

In yet another aspect of present invention, there is provided Crofelemer having a polydispersity index in the range of 0.9 to 1.2.

In yet another aspect of present invention, there is provided Crofelemer, wherein taspine is present at an amount of less than 500 ppm as measured by HPLC.

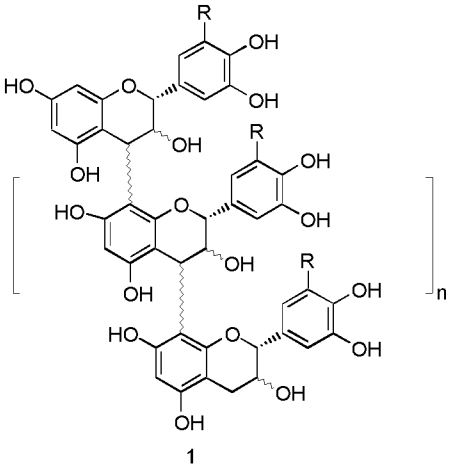
In yet another aspect of present invention, there is provided Crofelemer having less than 0.15% of an impurity. In one embodiment of this aspect Crofelemer has less than 0.15% of an impurity as measured at RRT 0.07.

In yet another aspect of present invention, there is provided Crofelemer having water content in the range of 7% to 17 % (wt %). Preferably the water content is analyzed by KF method.

In yet another aspect of present invention, there is provided Crofelemer having water content at about 7 to about 17 percent by weight, and having a total organic compound impurity content of less than about 1% as measured by HPLC.

In yet another aspect of present invention, there is provided Crofelemer containing less than 1000 ppm of any one of acetone, n-butanol, diacetone alcohol and any combinations thereof.

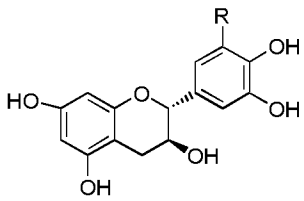
Another aspect of present invention provides amorphous Crofelemer having formula



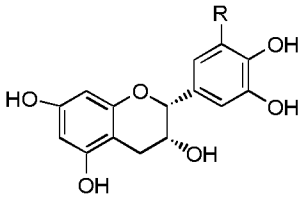
1

R= H (procyanidin) and/or R= OH (prodelphinidin)
Average n ~ 5 (7 units)

wherein, the structure of the monomeric units are



2 R= H, (+)-catechin
4 R= OH, (+)-gallocatechin



3 R= H, (-)-epicatechin
5 R= OH, (-)-epigallocatechin

Yet another aspect of present invention provides amorphous Crofelemer having water content from 7 to 17 percent by weight (preferably analyzed by KF method).

In yet another aspect of present invention, there is provided amorphous Crofelemer having:

i) a polydispersity index in the range of 0.9 to 1.2;

ii) taspine present in an amount at less than 500 ppm;

iii) an assay of greater than 85%;

iv) less than 0.15% of an impurity (preferably measured at RRT 0.07);

v) water content in the range of 7% to 17 % (wt %) (preferably as analyzed by KF method);

obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

The present invention also relates to the process for the production of purified proanthocyanidin polymeric compositions for use in pharmaceutically effective formulations. In particular, provided herein are processes for the production of a pure Crofelemer using column purification technique.

In one aspect of present invention, there is provided Crofelemer having

i) a polydispersity index in the range of 0.9 to 1.2;

ii) taspine present in an amount of less than 500 ppm;

iii) an assay of greater than 85%;

iv) less than 0.15% of an impurity (preferably measured at RRT 0.07);

v) water content in the range of 7% to 17 % (wt %) (preferably analyzed by KF method);

vi) in amorphous form

obtainable by a method comprising the steps of:

(a) providing a solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex;

(b) extracting the solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex with an organic solvent;

(c) separating the organic solvent and (d) concentrating aqueous layer to obtain solid; alternatively separating the aqueous layer and (d) concentrating organic solvent to obtain solid, liquid or concentrated syrup;

(e) dissolving the solid, liquid or concentrated syrup in water or water miscible solvents and removing the insoluble particulates if present from the solution;

(f) purifying the solution by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1; or by using two set column selected from CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1;

Another aspect of present invention provides a method of producing Crofelemer comprising the steps of:

(a) providing a solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex;

(b) extracting the solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex with an organic solvent;

(c) separating the organic solvent and (d) concentrating aqueous layer to obtain solid; alternatively separating the aqueous layer and (d) concentrating organic solvent to obtain solid, liquid or concentrated syrup;

(e) dissolving the solid, liquid or concentrated syrup in water or water miscible solvents and removing the insoluble particulates, if present, from the solution;

(f) purifying the solution by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1; or by using two set column selected from CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1;

Detailed Description of the Invention

U.S. Patent No. 5211944 (the US'944 patent) discloses, in detail, a method to isolate a proanthocyanidin polymer composition. The US'944 patent describes the isolation of an aqueous soluble proanthocyanidin polymer composition from *Croton spp.* and the use of the composition as an antiviral agent. According to the method disclosed in the US'944 patent, the desired proanthocyanidin polymer composition are obtained from various plants including but not limited to the classes Filices, Coniferae, Monocotyledoneae, and Dicotyledonae. They can be obtained using the entire tree or plant, the bark, stems, roots or latex. The example discloses proanthocyanidin polymer composition obtained from the latex of *Croton lechleri.*

U.S. patent No. 7325195 (the US'195) discloses a method to isolate a proanthocyanidin polymer composition from the *Croton lechleri* plant.

Unfortunately, these known method do not allow for the convenient, large scale, efficient and economical production of proanthocyanidin polymeric composition such as Crofelemer. Waste management is also now an important consideration as it has become an essential and fundamental part of the regulatory issues associated with the pharmaceutical and/or chemical industry worldwide. This creates a need for environmentally friendly processes for the the production of the same.

The inventors of the present invention have surprisingly found that the lyophilization of crude latex or addition of water to plant latex during isolation of partially purified Crofelemer has helped to increase yield and reduce the need for processing steps, which ultimately the reduces the cost of production of Crofelemer.

The starting material used for production herein was plant latex obtained from a from *Croton lechleri* trees. *C. lechleri* trees were tapped and felled near the village of San Pablo de Cuyana on the Nanay River 100 kilometers from Iquitos, Peru. The latex was obtained over a period of 24 hours by scoring the trees. The natural origin and source of starting material used herein is an important factor with respect to the cost of the production. The latex material must be transported to and within different countries (for example India) for the large scale production for pharmaceutical use. Thus, the latex is freeze dried to obtain freeze dried solid (powder) which can be more conveniently transported or crude latex plant can be used for production of Crofelemer.

The starting material used herein is plant latex which is obtained from the bark of *Croton lechleri.* The crude plant latex, partially purified plant latex, concentrated crude plant latex, or concentrated partially purified plant latex may comprising mud or may be freeze-dried (lyophilized) powder obtained from the plant latex.

As used herein, "mud" refers to sediment formed on storage. Crude plant latex can be obtained from the bark of *Croton lechleri.* This latex is collected and stored in barrels. On storage, sediment deposited is referred to as "mud". This mud is generally discarded.

As used herein, "freeze-drying" or "lyophilization" involves freezing solutions or suspensions of thermosensitive materials, followed by primary and secondary drying. The technique is based on sublimation of water at subzero temperature under vacuum without the solution melting.

The present invention relates to a process for the isolation and purification of a proanthocyanidin polymeric composition, the composition having high purity and being suitable for use in pharmaceutically effective formulations. In particular, the invention relates to an efficient, economical and/or environmentally friendly process for the production of Crofelemer suitable for pharmaceutical formulations and having a level of purity and concentration that enables it to be used in a therapeutically effective manner.

In one aspect, the present invention provides Crofelemer having a polydispersity index in the range of 0.9 to 1.2, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

In another aspect, the present invention provides Crofelemer wherein taspine is present in an amount of less than 500 ppm; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

In yet another aspect of present invention, there is provided Crofelemer having an assay of greater than 85%; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

In another aspect of present invention, there is provided Crofelemer having less than 0.15% of an impurity; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique. In one embodiment of this aspect Crofelemer has less than 0.15% of an impurity as measured at RRT 0.07.

In yet another aspect of present invention, there is provided Crofelemer having water content in the range of 7% to 17 % (wt %); obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique. In one embodiment of this aspect, the water content was analyzed by KF technique.

In yet another aspect, the present invention provides Crofelemer having a polydispersity index in the range of 0.9 to 1.2; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of present invention, there is provided Crofelemer, wherein taspine is present at an amount of less than 500 ppm; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of present invention, there is provided Crofelemer having an assay of greater than 85%; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In yet another aspect of present invention, there is provided Crofelemer having less than 0.15%; obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by a using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1. In one embodiment of this aspect Crofelemer has less than 0.15% of an impurity as measured at RRT 0.07.

In yet another aspect of present invention, there is provided Crofelemer having water content in the range of 7% to 17 % (wt %); obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is

in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1. In one embodiment of this aspect, the water content was analyzed by KF technique.

In yet another aspect of present invention, there is provided Crofelemer having purity of greater than about 98%.

In yet another aspect of present invention, there is provided Crofelemer having an assay of greater than about 85%.

In yet another aspect of present invention, there is provided Crofelemer having a polydispersity index in the range of 0.9 to 1.2.

In yet another aspect of present invention, there is provided Crofelemer wherein taspine is present at an amount of less than 500 ppm as measured by HPLC.

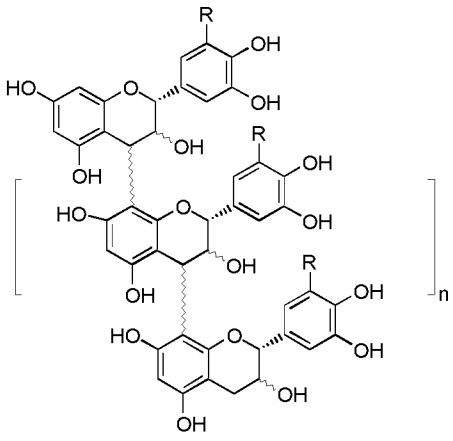
In yet another aspect of present invention, there is provided Crofelemer having less than 0.15% of an impurity (preferably measured at RRT 0.07).

In yet another aspect of present invention, there is provided Crofelemer having water content in the range of 7% to 17 % (wt %). Preferably the water content is analyzed by KF method.

In yet another aspect of present invention, there is provided Crofelemer having water content about 7 to about 17 percent by weight, and having a total organic compound impurity content less than about 1% as measured by HPLC.

In yet another aspect of present invention, there is provided Crofelemer containing less than 1000 ppm of any one of acetone, n-butanol, diacetone alcohol and combinations thereof.

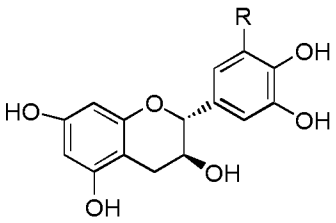
In yet another aspect of present invention, there is provided amorphous Crofelemer having formula



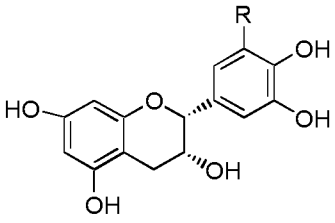
1

R= H (procyanidin) and/or R= OH (prodelphinidin)
Average n ~ 5 (7 units)

wherein, structure of monomeric units are



2 R= H, (+)-catechin
4 R= OH, (+)-gallocatechin



3 R= H, (-)-epicatechin
5 R= OH, (-)-epigallocatechin

In yet another aspect of present invention, there is provided amorphous Crofelemer having water content from 7 to 17 percent by weight. Preferably the water content is analyzed by KF method.

In yet another aspect of present invention, there is provided amorphous Crofelemer having:

a) a polydispersity index in the range of 0.9 to 1.2;

b) taspine present in an amount at less than 500 ppm;

c) an assay of greater than 85%;

d) less than 0.15% of an impurity (preferably measured at RRT 0.07);

e) water content in the range of 7% to 17 % (wt %) (preferably analyzed by KF method);

obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

Another aspect of the present invention provides a method for preparing Crofelemer comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a column chromatography technique.

In yet another aspect of present invention, there is provided a method for preparing Crofelemer comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using a single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or by using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

Yet another aspect of present invention provides a process for isolating partially purified Crofelemer (as described herein in step (A)) comprising the steps of:

(a) stirring mixture of crude plant latex or freeze-dried powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C;

(b) separating the liquid phase;

(c) optionally concentrating liquid phase to obtain solid, liquid or concentrated syrup; and

(d) optionally adding water or water miscible solvent(s) to the solid, liquid or concentrated syrup.

Method of isolating partially purified Crofelemer in step (A) may involve one or more embodiments. It is to be understood that the embodiments below are illustrative of the present invention and are not intended to limit the claims to the specific embodiments exemplified. It is also to be understood that the embodiments defined herein may be used independently or in conjunction with any definition, claim or any other embodiment defined herein. Thus the invention contemplates all possible combinations and permutations of the various independently described embodiments.

In one embodiment, the method involves isolating partially purified Crofelemer from crude plant latex.

In one embodiment, the method involves isolating partially purified Crofelemer from freeze-dried (lyophilized) plant latex;

In one embodiment, the step (a) comprises adding water or water miscible solvent(s) to crude plant latex or freeze-dried (lyophilized) plant latex. The resultant mixture can be stirred at a temperature in the range of 15°C to 60°C or more for a period of 5 minutes to about one or several hour(s). In this embodiment, preferably the mixture is warmed or heated at a temperature of about 35°C to 45°C for 30 to 60 minutes, most preferably at 40°C for 60 minutes. In this embodiment the isolation step may be optionally followed by separation, concentration and addition of water or water miscible solvent(s) to obtain the solid, liquid or concentrated syrup.

In one embodiment, the step (b) comprises separating the liquid phase using but not limited to the method(s) known in the art such as filtration, sedimentation, centrifugation and/or decantation or any combination thereof.

In one embodiment, step (b) separation can be done by filtration which may be performed using filtering aid. In this embodiment, the filtering material comprises one or more of diatomaceous earth, charcoal, bentonite, cellulose, glass, sand, or filter paper or commercially available filter (e.g., sparkler filter).

In one embodiment, step (b) separation can be done by sedimentation which may be performed by keeping the plant latex mixture undisturbed for one or more minutes and up to several hour(s) with or without cooling, preferably with cooling at a temperature in the range of 5°C to 15°C, preferably at 10°C. In this embodiment, the sediment may be allowed to settle for a time period of at least 1 hour, preferably more than one hour, more preferably the time period is between 10 to 20 hours, most preferably 15 hours.

In one embodiment, step (b) separation can be done using centrifugation technique. Centrifugation of the solution, suspension or mixture can be done at a cooling temperature such as at 10°C using a speed of 100 to 10,000 revolution per minutes (RPM), preferably using any speed in the range of 2000 RPM to 4000 RPM, more preferably using 3000 RPM followed by decantation.

In one embodiment, step (b) separation can be done using one or any combination of method(s) known in the art and independently selected from filtration, sedimentation, centrifugation and/or decantation as discussed above. In this embodiment preferably the separation using any combination of method(s) can be performed in the sequence of filtration, sedimentation followed by centrifugation and decantation.

In one embodiment, in step (c) the liquid phase is concentrated to obtain concentrated syrup, liquid or solid, which can be used in the next step. In this embodiment, the concentration of liquid phase may be done using but not limited to the processes or methods or combination thereof known in the art such as ultrafiltration and /or by using rotary evaporator or combination(s) thereof. In this embodiment, preferably the methods used are ultrafiltration followed by rotary evaporation.

In one embodiment, ultrafiltration is performed with a semi-permeable membrane.

In one embodiment, the semi-permeable membrane permits passage of solutes with molecular weight in the range of 1 to 1000 Da. In this embodiment it is preferred that the semi-permeable membrane permits passage of solutes up to a molecular weight selected from the group consisting of 500 Da and 1 kDa, and is preferably a semi-permeable membrane that permits passage of solutes up to a molecular weight of 1 kDa.

In one embodiment, concentration and drying can be done preferably using a rotary evaporator at a temperature of approximately 37°C (±2°C) or any other temperature or using any other suitable drying methodologies including, but are not limited to, tray drying or spray drying.

In one embodiment, the processing of step (c), which is concentration and drying to obtain concentrated syrup or liquid or solid form, can be selected from the group consisting of ultrafiltration, followed by freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, tray drying, spray drying, and any combinations thereof.

In one embodiment, wherein in step (d) water or water miscible solvent(s) (e.g., approximately 5 mL per gram of latex) may be added to the concentrated syrup or liquid or solid, with or without mixing, preferably with mixing to obtain the desired solution which may or may not contain insoluble particulate matter. This solution may be filtered if insoluble particulates present in solution. Filtration may be performed by using filtering aid. In this embodiment, the filtering material comprises one or more of diatomaceous earth, charcoal, bentonite, cellulose, glass, sand, or filter paper or commercially available filter (e.g., sparkler filter).

Method of purification of partially purified Crofelemer in step (B) may involve one or more embodiments. It is to be understood that the embodiments below are illustrative of the present invention and are not intended to limit the claims to the specific embodiments exemplified. It is also to be understood that the embodiments defined herein may be used independently or in conjunction with any definition, claim or any other embodiment defined herein. Thus the invention contemplates all possible combinations and permutations of the various independently described embodiments.

In one embodiment, purification may be carried out by using chromatographic techniques such as but not limited to ion exchange chromatography and/or size exclusion chromatography or combination thereof using water and/or water miscible solvent as eluent. In this embodiment the preferred chromatographic technique used is ion exchange chromatography by using adsorbent, martial, matrix or resin designed or may used from the commercial source for the same; more preferably, the resin used herein is CM-Sepharose.

In one embodiment the size exclusion chromatography is Sephadex LH-20.

The column purification can be done by using a single column of CM-Sepharose Fast Flow Column or by using two set columns of CM-Sepharose Fast Flow Column and Sephadex LH-20. Two set column when used can be used jointly in a series or separately.

In one embodiment, purification performed using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1.

In one embodiment, purification performed using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1 .

In one embodiment, the ratio of CM-Sepharose to the feed in a column is at least about 3.5:1, more preferably in the ratio of at least 4:1 (for e.g., 4 mL or 4 gm of CM-Sepharose for 1 gm of the plant latex feed). In preferred embodiment, the ratio of CM-Sepharose to the feed in a column is at least about 6:1 (for e.g., 6 mL or 6 gm of CM-Sepharose for 1 gm of the plant latex feed).

In one embodiment, the ratio of CM-Sepharose to the feed in a column is in the range of about 3.5:1 to 11:1, preferably ratio is in the range of about 4:1 to 9:1.

In one embodiment, the ratio of CM-Sepharose to the feed in a column is less than 11:1, preferably the ratio is less than 9:1 .

In one embodiment, the CM-Sepharose suspension may be filled in to the column to obtain bed volume of at least about 35 mL (for 10 gm of solid plant latex feed or substrate), preferably at least about 40.0 mL per 10 gm of the plant latex feed or substrate.

In one embodiment, the CM-Sepharose may be filled in to the column to obtain bed width (column inner diameter) of at least about 3.2 cm (for 10 gm of solid plant latex feed or substrate), preferably at least 3.4 cm per 10 gm of plant latex feed.

In one embodiment, the CM-Sepharose suspension may be filled in to the column to obtain appropriate bed height (or column length) of at least about 4.2 cm (for 10 gm of solid plant latex feed or substrate), preferably at least 4.5 cm per 10 gm of plant latex feed or substrate.

In one embodiment, the ratio of Sephadex LH-20 to the feed in a column is at least about 8:1 to 90:1, more preferably in the ratio of at least 12:1 to 90:1 (e.g., 12 gm of CM-Sepharose for 1 gm of the plant latex feed).

In one embodiment, the Sephadex LH-20 filled in to the column to obtain column length or bed height of at least about 8 cm (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 13 cm per 10 gm of substrate or feed.

In one embodiment, the Sephadex LH-20 filled in to the column to obtain column inner diameter or bed width of at least about 3.2 cm (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 3.4 cm per 10 gm of substrate or feed.

In one embodiment, the Sephadex LH-20 filled in to the column to obtain appropriate bed volume of at least about 110 mL (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 118 mL per 10 gm of solid or feed.

In one embodiment, the material loaded on to the ion exchange column (for e.g., CM-Sepharose) and the column is washed with purified water. Then the material is eluted from the column with an aqueous acetone solution, thereby loading the material onto the size exclusion column (for e.g., Sephadex LH-20).

The size exclusion column may be then disconnected from the ion exchange resin column and the material is eluted off with an aqueous acetone solution.

Eluents may be selected from water and water miscible solvent(s) and combination thereof. The preferred eluent used herein is water followed by an aqueous acetone solution (preferably 50% acetone in water, more preferably 45% acetone in water, most preferably 30% acetone in water).

The fractions are collected and monitored with a detector. Fractions containing proanthocyanidin polymers (e.g., Crofelemer) material are combined. The proanthocyanidin polymers (e.g., Crofelemer) produced as disclosed herein can be analyzed or detected by any methods known in the art. For example, proanthocyanidin polymers can be detected by ultraviolet absorbance (lambda-max). Certain proanthocyanidin monomers and polymers, for example, have broad peaks around 200 to

about 300 nm, for example between about 200 and about 215 nm (e.g., about 205 - 210 nm) and between about 260 and about 295 nm (e.g., about 275 - 280 nm). Fractions containing proanthocyanidin polymers can have additional major UV absorption maxima from about 400 nm to about 500 nm.

In one embodiment, the concentration of eluent may be done by using but not limited to the processes or methods or combination thereof known in the art such as ultrafiltration or by using rotary evaporator or combination thereof.

In one embodiment, the ultrafiltration is performed with a semi-permeable membrane.

In one embodiment, the semi-permeable membrane permits passage of solutes with molecular weight in the range of 1 to 1000 Da. In this embodiment the semi-permeable membrane permits passage of solutes up to a molecular weight selected from the group consisting of 500 Da and 1 kDa, preferably semi-permeable membrane that permits passage of solutes up to a molecular weight of 1 kDa.

Preferably concentration followed by drying can be done using rotary evaporator at a temperature of approximately 37°C (±2°C) or any other temperature or any other suitable drying methodologies include, but are not limited to, tray drying or spray drying.

In one embodiment, the processing of concentration and drying to obtain solid can be selected from the group consisting of ultrafiltration, freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, tray drying, spray drying, and combinations thereof.

Processes disclosed herein provide Crofelemer comprising purity of greater than about 90% (by chromatographic purity which, may be for example determined by the analysis of detectable components compared to a reference standard using chromatography), preferably greater than about 95%, preferably greater than about 98%, preferably greater than about 99%, more preferably greater than about 99.9%, and most preferably greater than about 99.95%. For example, the purity of Crofelemer is about 98% to about 99.9%, or about 99.5% to about 99.99%.

In a preferred embodiment, Crofelemer produced according to the invention has a lower concentration of taspine than the concentration of taspine in the starting material (e.g., plant latex). For example, the amount of taspine which is present in the original

latex can be reduced through the process according to the invention. Taspine levels in the Crofelemer produced according to the invention may range from 1% by chromatographic purity down to below detectable limits. For example, the upper level for the amount of taspine in the Crofelemer may be 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0.05% by chromatographic purity, or any amount in between or below the listed amounts down to the limit of detectability. In a preferred embodiment, taspine levels are below 0.1% by chromatographic purity (i.e. 1000 ppm) in Crofelemer. Crofelemer has 0.1% by chromatographic purity taspine and 0% by chromatographic purity taspine (i.e. no detectable amount of taspine) when produced according to the process of the invention. In one preferred embodiment, Crofelemer having taspine levels less than 500 ppm.

In one embodiment, the invention includes Crofelemer having total organic compound impurity content less than about 1 area-percent by high performance liquid chromatography.

In one embodiment, the invention includes Crofelemer with increased homogeneity. For example, in one embodiment, Crofelemer having a polydispersity index in the range of 0.9 to 1.2.

In one embodiment, the invention includes amorphous Crofelemer having water content about 7 to about 17 percent by weight, and having total organic compound impurity content less than about 1 area-percent by high performance liquid chromatography.

In one embodiment, there is provided Crofelemer obtained has less than 0.15% of an impurity. Preferably Crofelemer obtained has less than 0.15% of impurity measured at RRT 0.07.

It is known by those skilled in the art, the management of process impurities is greatly enhanced by understanding their chemical structures and synthetic pathways, and by identifying the parameters that influence the amount of impurities in the final product.

Impurities are identified spectroscopically and by other physical methods, and then the impurities are associated with a peak position in a chromatogram (or a spot on a TLC plate). Thereafter, the impurity can be identified by its position in the chromatogram, which is conventionally measured in minutes between injection of the

sample on the column and elution of the particular component through the detector, known as the "retention time" ("rt"). This time period varies based upon the condition of the instrumentation and many other factors. To mitigate the effect that such variations have upon accurate identification of an impurity, practitioners use "relative retention time" ("RRT") to identify impurities.

In another aspect of present invention, Crofelemer having water content about 7 to about 17 percent by weight, and having a total organic compound impurity content less than about 1% by HPLC.

In another aspect of present invention, Crofelemer containing less than 1000 ppm of any one of acetone, n-butanol, diacetone alcohol or any combination(s) thereof.

The present invention also relates to substantially pure proanthocyanidin polymer composition. For the purposes of this invention, substantially pure is greater than about 98% pure, preferably, proanthocyanidin polymer composition of the present invention is greater than about 99% pure.

In an embodiment, the present invention relates to process for producing substantially pure Crofelemer to be used for therapeutically effective pharmaceutical composition. The Crofelemer is preferably prepared from latex from *Croton spp,* preferably *Croton lechleri.*

The isolation and purification of proanthocyanidin polymer composition in a desired purity is challenging due to the similar chemical properties of many other isomers, as well as their related impurities.

The inventors of the present application have found that the repetition of the prior art method does not yield the desired proanthocyanidin polymer composition with high purity. The inventors of the present invention have overcome the problem associated with the isolation of pure Crofelemer with an alternative process for the isolation and purification of Crofelemer.

The isolation of Crofelemer primarily involves purification on a two column system, wherein the material is first fed onto a chromatographic system consisting of a ion-exchange resin which is further connected to a column consisting of size-exclusion resin.

The ion-exchange resin, such as CM-Sepharose Fast Flow, serves to remove cationic impurities from the material, while the size-exclusion resin, such as LH-20, purifies the polymer composition and delivers the composition in desired molecular weight range.

Surprisingly, the inventors of the present application have found that the efficient mixing of the initial material with the suitable solvent system which may include water or water in combination with water miscible solvent and then passing it through the two column system provides the Crofelemer in high purity and better analytical profile.

It was found that incomplete mixing of the starting material, before loading onto a column results into inefficient utilization of the resin bed. Further it was found that insoluble particulates present in the feed solution choked the column and affected the efficiency of the resin considerably. It was found that due to presence of insoluble particulates, the feed solution failed to contact many active sites of the resin. Thus failure to achieve minimum contact time may result in the passage of cationic impurities such as taspine and other impurities through the column to the next level.

To overcome the above stated problems, the inventors of the present application developed a process which involves dissolving the initial material in a suitable solvent system such as water and filtered through a sparkler filter to remove the insoluble particulates. The feed solution which is substantially free of insoluble particulates is then applied into a two stage chromatography system.

For the efficient functioning of the column system, it is required that the column system remains in 'wet' condition. The drying of the column may result into cracking the column composition, and it has been found that this may lead to getting proanthocyanidin polymer composition with less purity.

In one aspect of present invention, the proanthocyanidin polymer composition (for e.g., Crofelemer) is isolated in a highly pure state by the method which comprises:

(a) providing a solution of plant latex;

(b) adding an organic solvent(s) to the solution of plant latex;

(c) separating the organic solvent and concentrating aqueous layer to obtain a solid; alternatively

(c) separating the aqueous layer and concentrating organic solvent to obtain a solid;

(d) dissolving the solid in an aqueous solvent;

(e) removing the insoluble particulates from the solution (d);

(f) subjecting the solution to chromatography; and

(g) isolating proanthocyanidin polymer composition.

In one embodiment, Crofelemer is isolated from *Croton lechleri.* In another embodiment, Crofelemeris isolated from *Calophyllum inophylum.* The latex material may contain mud also which may be optionally removed.

In one embodiment, the latex is kept below room temperature (for e.g., below 25°C) for a period of time to allow sediment to settle which is for example, from about 1 hour to about 30 days. The latex is then mixed with water below room temperature (for e.g., at 2-8°C) and allowed to settle for at least 12 hours or may be mixed with methyl ethyl ketone. The sedimented solid material is discarded and the supernatant is collected. Optionally the supernatant may be passed through a filter to remove the solid material. The operation may be carried out below room temperature.

In one embodiment, the content of the step (a) wherein water is used, is mixed with a miscible or immiscible solvent(s) selected from methanol, ethanol, propanol, butanol, pentanol, hexanol, ethylene glycol, propylene glycol, ethyl acetate, dichloromethane, trichloromethane, tetrachloromethane, dichloroethane, diethyl ether, acetone, dimethylformamide, dimethylsulfoxide, ether, mixtures thereof, and the like. The aqueous layer was preferably mixed with immiscible solvent. In one embodiment, the immiscible solvent is n-butanol. The aqueous layer was washed successively with n-butanol.

In one embodiment, the layer containing Crofelemer may be processed by ultrafiltration followed by evaporation with or without heat, evaporation with or without vacuum, freeze drying, spray drying, and the like, including combinations of processing techniques, to yield solid.

In one embodiment, the solid obtained in step (c) is mixed with a suitable solvent such as water or mixture of water and water miscible solvent. In a preferred embodiment, the solvent is water.

The insoluble particulates are then removed by filtering the solution through an appropriate medium such as sparkler filter.

In one embodiment, the obtained solution in step (e) is subjected to column chromatography.

In one embodiment, the isolation of Crofelemer primarily involves purification on a two column system, wherein the material is first fed onto a chromatographic system consisting of an ion-exchange resin which is further connected to a column consisting of size-exclusion resin.

In one embodiment, the ion exchange resin may be CM-Sepharose, which is a carboxy-methyl modified agarose.

In one embodiment, the column used in chromatography comprises a single column of CM-Sepharose Fast Flow Column or two set column of CM-Sepharose Fast Flow Column and Sephadex LH-20.

In one embodiment, the elution of the solid phase was carried out with a solvent system selected from the group consisting of water, acetone, methanol, ethanol, glycol and mixtures thereof. In a preferred embodiment, the eluent used is water.

In one embodiment, the material is loaded on to the ion exchange column and the column is washed with purified water. Then the material is eluted from the column with an aqueous acetone solution, thereby loading the material onto the size exclusion column.

In one embodiment, the second column consists of size-exclusion resin which is for example Sephadex LH-20, which is a hydroxypropylated cross-linked dextran. The size exclusion column is then disconnected from the ion exchange resin column and the material is eluted off with an aqueous acetone solution. The fractions are collected and monitored with a detector. Fractions containing Crofelemer material are combined. In the next step, Crofelemer is isolated in solid form.

In one embodiment, the isolation can be done by processing which is selected from the group comprising of ultrafiltration, freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, spray drying, and combinations thereof.

The Crofelemer produced as disclosed herein can be analyzed by any methods known in the art. For example, Crofelemer can be detected by ultraviolet absorbance

(lambda-max). Crofelemer has broad peaks around 200 to about 300 nm, for example between about 200 and about 215 nm (e.g., about 205 - 210 nm) and between about 260 and about 295 nm (e.g., about 275 - 280 nm). Fractions containing Crofelemer can have additional major UV absorption maxima from about 400 nm to about 500 nm, from between 425 and 475 nm, and about 460 nm.

In one embodiment, the highly pure Crofelemer has a purity of greater than about 98%, specifically greater than about 99%, more specifically greater than about 99.9%, and most specifically greater than about 99.95% as measured by HPLC. For example, the purity of the Crofelemer is about 98% to about 99.9%, or about 99.5% to about 99.99%.

In one embodiment, Crofelemer disclosed herein having an assay of greater than about 85%.

The molecular weight range and distribution of a polymer is generally established using Gel Permeation chromatography (GPC) and characterized by the number average molecular weight (Mn), weight average molecular weight (Mw) and the polydispersity index. The polydisepersity index is a measure of breadth of the molecular weight distribution.

In one embodiment, the invention includes Crofelemer with increased homogeneity. For example, in one embodiment, Crofelemer prepared has a polydispersity index in the range of 0.9 to 1.2.

In one embodiment, Crofelemer produced according to the invention has a lower concentration of taspine than the concentration of taspine in the latex starting material. For example, the amount of taspine which is present in the original latex can be reduced through the process according to the invention. Taspine levels in the Crofelemer produced according to the invention may range from 1% by chromatographic purity down to below detectable limits. For example, the upper level for the amount of taspine in the Crofelemer may be 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0.05% by chromatographic purity, or any amount in between or below the listed amounts down to the limit of detectability. In a preferred embodiment, taspine levels are below 0.05% by chromatographic purity (i.e. 500 ppm) in Crofelemer.

According to one embodiment, there is provided a Crofelemer obtained with less than 0.15% of an impurity (preferably measured at RRT 0.07).

According to one embodiment, the impurity measured at RRT 0.07 may be used as a reference marker for determining the purity of Crofelemer and a residual amount of water.

According to one embodiment, there is provided Crofelemer, wherein, the water content is about 7-17% (wt %). Preferably the water content is analyzed by KF method.

According to one embodiment, wherein the Crofelemer is dried under vacuum.

According to one embodiment, wherein the Crofelemer is dried under vacuum, and the temperature for vacuum drying is lower than 40 degrees centigrade.

In a preferred embodiment, the temperature for vacuum drying is 20-35°C.

In yet another aspect of the present invention, the Crofelemer is in amorphous form.

In yet another aspect of the present invention, amorphous Crofelemer having water content about 7 to about 17 percent by weight.

In yet another aspect of the present invention, amorphous Crofelemer having water content about 7 to about 17 percent by weight, and having a total organic compound impurity content less than about 1 area-percent by HPLC.

In yet another aspect of the present invention, Crofelemer containing about 50 ppm to about 1000 ppm of any of the residual solvent. In a preferred embodiment, Crofelemer containing about 100 ppm to about 1000 ppm of any one of acetone, n-butanol, diacetone alcohol (i.e., 4-hydroxy-4-methyl-2-pentanone).

The present invention also relates to the process for the production of purified proanthocyanidin polymeric compositions for use in pharmaceutically effective formulations. In particular, provided herein are processes for the production of a pure Crofelemer using column purification technique.

U.S. patent No. 7325195 discloses a method to isolate the proanthocyanidin polymer composition from the *Croton lechleri* plant. The latex of *Croton lechleri* is mixed with purified water and then any insoluble material in the latex solution is allowed to settle. The supernatant is pumped away from the residue and then extracted with *n*-butanol several times. After each extraction, the alcohol phase is discarded and the aqueous phase retained. The aqueous phase is concentrated, for example, using an ultrafiltration device with a 1 kD cut-off membrane. The retentate from the ultrafiltration

is then concentrated to dryness, for example using tray-dryers at approximately 37°C (±2°C). The dried material is subsequently dissolved in water and then chromatographed on a two column system, wherein the material is run over a CM-Sepharose and then an LH-20 column in a series. Specifically, the dissolved material is loaded onto the cation exchange column and then washed with purified water. The proanthocyanidin polymer material is eluted from the cation exchange column with an aqueous acetone solution (preferably 30% acetone), thereby loading the proanthocyanidin polymer material onto the sizing column. The fractions are collected and monitored with a UV detector, for e.g., at a wavelength of 460 nm. Fractions containing the proanthocyanidin polymer material are combined and concentrated, for example, by ultrafiltration using, for e.g., a 1 kD cut-off membrane (as described above for the ultrafiltration step prior to the chromatography steps). The retentate may then be concentrated to dryness using a suitable drying method, such as but not limited to a rotary evaporator, at a temperature of approximately 37°C (±2°C). Other suitable drying methodologies include, but are not limited to, tray drying and spray drying.

The inventors of the present application have found that the repetition of the prior arts method does not yield the desired proanthocyanidin polymer composition with high purity. The problem associated in obtaining the desired product was studied and was found to be related to the column purification, column bed height and ratio of feed to chromatographic material used for column filling.

Inventors of the present invention optimized the column length (bed height), column inner diameter (bed width) and column volume (bed volume) in relation to obtain the desired Crofelemer with high purity and consistency to be used for therapeutically effective pharmaceutical compositions.

The proanthocyanidin polymer composition may contain undesired taspine in high amount. Taspine is an alkaloid also found in *Croton* species and has been reported useful as anti-inflammatory composition, but has other side effects which makes it undesirable. Thus it is desirable to keep its content to minimum level. Also it is desirable to develop a process which would minimize the content of taspine in the final proanthocyanidin polymer composition.

The ion-exchange resin, such as CM-Sepharose Fast Flow, serves to remove cationic impurities such as taspine from the material, while the size-exclusion resin, such as LH-20, purifies the polymer composition and delivers the composition in desired molecular weight range.

In one aspect of present invention, Crofelemer having

i) a polydispersity index in the range of 0.9 to 1.2;

ii) taspine in an amount of less than 500 ppm;

iii) an assay of greater than 85%;

iv) less than 0.15% of an impurity (preferably measured at RRT 0.07);

v) water content in the range of 7-17 % (wt %) (preferably analyzed by KF method);

vi) amorphous form;

obtainable by a method comprising the steps of:

(a) providing a solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex;

(b) extracting the solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex with an organic solvent(s);

(c) separating the organic solvent and (d) concentrating aqueous layer to obtain solid, liquid or concentrated syrup; alternatively separating the aqueous layer and (d) concentrating organic solvent to obtain solid, liquid or concentrated syrup;

(e) dissolving the solid, liquid or concentrated syrup in water or water miscible solvents and removing the insoluble particulates if present from the solution;

(f) purifying the solution by using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1; or two set column selected from CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1;

In another aspect of present invention, a method of producing Crofelemer comprising the steps of:

(a) providing a solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex;

(b) extracting the solution of crude plant latex or freeze-dried (lyophilized) powder of plant latex with an organic solvent(s);

(c) separating the organic solvent and (d) concentrating aqueous layer to obtain solid, liquid or concentrated syrup; alternatively separating the aqueous layer and (d) concentrating organic solvent to obtain solid, liquid or concentrated syrup;

(e) dissolving the solid, liquid or concentrated syrup in water or water miscible solvents and removing the insoluble particulates if present from the solution;

(f) purifying the solution by using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1; or two set column selected from CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1;

The starting material, crude plant latex or freeze-dried (lyophilized) powder of plant latex is from a *Croton spp.* or *Calophyllum spp.* The crude plant latex, partially purified plant latex, concentrated crude plant latex, or concentrated partially purified plant latex may comprising the mud obtained from the plant latex.

In one embodiment, the Crofelemer is from *Croton lechleri.* In another embodiment, the Crofelemer is from *Calophyllum inophylum.* The latex material may contain mud also which may be optionally removed.

As used herein, mud refers to sediment formed on storage. Crude plant latex can be obtained from the bark of *Croton lechleri.* This latex is collected and stored in barrels. On storage, sediment deposited is referred as "Mud". This mud is generally discarded.

In one embodiment, wherein in step (a) the latex is kept below room temperature (for example, below 25°C) for a period of time to allow sediment to settle which is for example, from about 1 hour to about 30 days. The latex is then mixed with water below room temperature (for example at 2-8°C) and allowed to settle for at least 12 hours or may be mixed with methyl ethyl ketone. The sedimented solid material is discarded and

the supernatant is collected. Optionally the supernatant may be passed through a filter to remove the solid material. The operation may be carried out below room temperature.

In one embodiment, wherein in step (b) the red supernatant solution from step (a) is extracted with a water miscible or immiscible solvent(s) selected from methanol, ethanol, propanol, butanol, pentanol, hexanol, ethylene glycol, propylene glycol, ethyl acetate, dichloromethane, trichloromethane, tetrachloromethane, dichloroethane, diethyl ether, acetone, dimethylformamide, dimethylsulfoxide, ether, mixtures thereof The aqueous layer was preferably mixed with immiscible solvent. In one embodiment, the immiscible solvent is w-butanol. The aqueous layer is washed successively with *n*-butanol.

In one embodiment, wherein in step (c), the layer containing Crofelemer may be processed by ultrafiltration followed by stage (d) evaporation with or without heat, evaporation with or without vacuum, freeze drying, spray drying, and the like, including combinations of processing techniques, to yield solid, liquid or concentrated syrup.

In one embodiment, the step (c) comprises of separating the aqueous layer by using but not limited to the method(s) known in the art such as filtration, sedimentation, centrifugation and/or decantation or combination thereof.

In one embodiment, step (c) separation can be done by filtration which may be performed by using filtering aid. In this embodiment, the filtering material comprises one or more of diatomaceous earth, charcoal, bentonite, cellulose, glass, sand, or filter paper or commercially available filter (e.g., sparkler filter).

In one embodiment, step (c) separation can be done by sedimentation which may be performed by keeping the plant latex mixture undisturbed for couple of minutes to several hour(s) with or without cooling, preferably with cooling in the temperature range of 5°C to 15°C, preferably at 10°C. In this embodiment, the sediment may be allowed to settle for time period of at least 1 hour or more, preferably more than one hour, more preferably time period is between 10 to 20 hours, most preferably 15 hours.

In one embodiment, step (c) separation can be done by centrifugation technique. Centrifugation of the solution, suspension or mixture can be done at cooling temperature such as at 10°C using speed of 100 to 10,000 revolutions per minute (RPM), preferably

using any speed in the range of 2000 RPM to 4000 RPM, more preferably using 3000 RPM followed by decantation.

In one embodiment, step (c) separation can be done by using one or combination of more than one method(s) known in the art and independently selected from the group of filtration, sedimentation, centrifugation and/or decantation as discussed above. In this embodiment preferably the separation using combination of method(s) can be performed in the sequence of filtration, sedimentation followed by centrifugation and decantation.

In one embodiment, the step (d) is concentration of aqueous layer to obtain solid, liquid or concentrated syrup which can be used in the next step. In this embodiment, the concentration of aqueous layer may be done by using but not limited to the processes or methods or combination thereof known in the art such as ultrafiltration and/or by using rotary evaporator or combination thereof. In this embodiment preferably, methods used are ultrafiltration followed by rotary evaporation.

In one embodiment, the ultrafiltration is performed with a semi-permeable membrane.

In one embodiment, the semi-permeable membrane permits passage of solutes with molecular weight in the range of 1 to 1000 Da. In this embodiment preferred the semi-permeable membrane permits passage of solutes up to a molecular weight selected from the group consisting of 500 Da and 1 kDa, preferably semi-permeable membrane that permits passage of solutes up to a molecular weight of 1 kDa.

In one embodiment, concentration and drying can be done preferably using rotary evaporator at a temperature of approximately 37°C (±2°C) or any other temperature or any other suitable drying methodologies that include, but are not limited to, tray drying or spray drying.

In one embodiment, the processing of step (d), which is concentration and drying to obtain solid, liquid or concentrated syrup can be achieved by performing the techniques selected from the group consisting of ultrafiltration, followed by freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, tray drying, spray drying, and combinations thereof.

In the step (e), the solid referred herein as "feed" or concentrated syrup or liquid is mixed with a suitable solvent such as water or mixture of water and water miscible

solvent, with or without mixing, preferably with mixing to obtain desired solution which may or may not contain insoluble particulate matter.

In a preferred embodiment, 10 gram solid plant latex is mixed with approximately 125 mL of water.

As used herein, the water miscible solvent(s) used is preferably alcohol of 1-3 carbon atoms (e.g., ethanol) or acetone.

The insoluble particulates if present may be then removed by filtering the solution through an appropriate medium such as sparkler filter.

In the next step, the obtained solution in step (e) is subjected to column chromatography.

In one embodiment, the purification is performed on two column system, wherein the material is first loaded onto a chromatographic system consisting of an ion-exchange resin (e.g., CM-Sepharose Fast Flow Column) which is further connected to a column consisting of size-exclusion resin (e.g., Sephadex LH-20).

In one embodiment, the purification is performed on single column system of an ion-exchange resin (e.g., CM-Sepharose Fast Flow Column).

In one embodiment, the ion exchange resin or medium may be CM-Sepharose, which is a carboxy-methyl modified agarose.

In one embodiment, the size-exclusion resin or medium may be Sephadex LH-20, which is a hydroxypropylated cross-linked dextran.

In one embodiment, the ratio of CM-Sepharose to the feed in a column is at least about 3.5:1, more preferably in the ratio of at least 4:1 (for e.g., 4 mL or 4 gm of CM-Sepharose for 1 gm of the plant latex feed).

In one embodiment, the ratio of CM-Sepharose to the feed in a column is at least about 6:1 (e.g., 6 mL or 6 gm of CM-Sepharose for 1 gm of the plant latex feed).

In one embodiment, the ratio of CM-Sepharose to the feed in a column is in the range of about 3.5:1 to 11:1, preferably ratio is in the range of about 4:1 to 9:1.

In one embodiment, the ratio of CM-Sepharose to the feed in a column is less than 11:1, preferably the ratio is less than 9:1 .

In one embodiment, the CM-Sepharose suspension may be filled in to the column to obtain bed volume of at least about 35 mL (for 10 gm of solid plant latex feed or

substrate), preferably at least about 40.0 mL per 10 gm of the plant latex feed or substrate.

In one preferred embodiment, the CM-Sepharose suspension may be filled in to the column to obtain bed volume of at least about 60 mL for 10 gm of solid plant latex feed or substrate.

In one embodiment, the CM-Sepharose may be filled in to the column to obtain bed width (column inner diameter) of at least about 3.2 cm (for 10 gm of solid plant latex feed or substrate), preferably at least 3.4 cm per 10 gm of plant latex feed.

In one embodiment, the CM-Sepharose suspension may be filled in to the column to obtain bed height such as but not limited to bed height (or column length) of at least about 4.2 cm (for 10 gm of solid plant latex feed or substrate), preferably at least 4.5 cm per 10 gm of plant latex feed or substrate.

In one embodiment, the ratio of Sephadex LH-20 to the feed in a column is at least about 8:1 to 90:1, more preferably in the ratio of at least 12:1 to 90:1 (e.g., 12 gm CM-Sepharose for 1 gm of the plant latex feed).

In one embodiment, the Sephadex LH-20 filled in to the column to obtain column length or bed height of at least about 8 cm (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 13 cm per 10 gm of substrate or feed.

In one embodiment, the Sephadex LH-20 filled in to the column to obtain column inner diameter or bed width of at least about 3.2 cm (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 3.4 cm per 10 gm of substrate or feed.

In one embodiment, the Sephadex LH-20 filled in to the column to obtain bed (column) volume of at least about 110 mL (for 10 gm solid plant latex feed or substrate used in ion exchange column), preferably at least 118 mL per 10 gm of solid or feed.

In one embodiment, the material loaded on to CM-Sepharose column and the column is washed with purified water. Then the material is eluted from the column with an aqueous acetone solution, thereby loading the material onto the Sephadex LH-20.

The Sephadex LH-20 column may be then disconnected from the CM-Sepharose column and the material is eluted off with an aqueous acetone solution.

In one embodiment, the chromatographic purification can be done by using eluent(s) which are selected from water and water miscible solvent(s) and combination thereof. The preferred eluent used herein is water followed by an aqueous acetone solution (preferably 50% acetone in water and/or less than 50%, more preferably 45% acetone in water and/or less than 45%, and most preferably 45% acetone in water and/or 30% acetone in water or combination thereof).

The fractions are collected and monitored with a detector. The Crofelemer produced as disclosed herein can be analyzed or detected by any methods known in the art. For example, Crofelemer can be detected by ultraviolet absorbance (lambda-max). Crofelemer has broad peaks around 200 to about 300 nm, for example between about 200 and about 215 nm (e.g., about 205 - 210 nm) and between about 260 and about 295 nm (e.g., about 275 - 280 nm). Fractions containing Crofelemer have additional major UV absorption maxima from about 400 nm to about 500 nm, from between 425 and 475 nm, and about 460 nm.

In the next step, Crofelemer is isolated in solid form.

In one embodiment, the isolation can be done by the processing which is selected from the group consisting of ultrafiltration, freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, spray drying, and combinations thereof.

In one embodiment, wherein the Crofelemer is dried under vacuum.

In one embodiment, wherein Crofelemer is dried under vacuum and the temperature for vacuum drying is lower than 40°C. In a preferred embodiment, the temperature for vacuum drying is 20-35°C.

In one embodiment, taspine level is below 2000 ppm by HPLC in the Crofelemer, when produced according to the process of the invention. For example, the upper level for the amount of taspine in the Crofelemer may be 2000 ppm, 1500 ppm, 1000 ppm, 500 ppm, 400 ppm, 300 ppm, 200 ppm, 100 ppm, or 50 ppm by HPLC, or any amount in between or below the listed amounts down to the limit of detection.

In one embodiment, the pure Crofelemer disclosed herein has purity of greater than about 90%, specifically greater than about 95%, more specifically greater than about 99%, and most specifically greater than about 99.5% as measured by HPLC. For

example, the purity of the Crofelemer is about 90% to about 95%, or about 99% to about 99.5% or more.

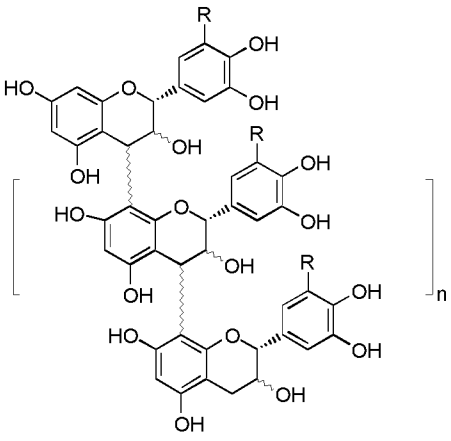
In one embodiment, Crofelemer disclosed herein having an assay of greater than about 85% and more specifically greater than about 90%.

In one embodiment, the Crofelemer disclosed herein has an average molecular weight of between about 1100 Da (Daltons) to about 3000 Da, or for example a molecular weight of between about 2000 Da to about 2500 Da; or for example a molecular weight of between about 1500 Da to about 3000 Da. and a polydispersity in the range of 0.5 to 1.8, or for example, in the range of 0.9 to 1.2, or in the range of 0.5 to 1.5, or in the range of 0.8 to 1.2. In one preferred embodiment, the Crofelemer has polydispersity index in the range of 0.9 to 1.20.

Examples of water miscible solvent(s) include, but are not limited to methanol, n-propanol, isopropanol, ethanol, dioxane, dimethyl sulphoxide, dimethyl formamide, tetrahydrofuran, acetone, acetic acid or acetonitrile.

Proanthocyanidin are a group of condensed tannins. The proanthocyanidin comprise monomeric units of leucoanthocyanidins. Leucoanthocyanidins includes catechins, epicatechins, gallocatechins, galloepicatechins, flavanols, flavonols, flavan-3,4-diols, leucocyanidins and anthocyanidins. In one embodiment, the proanthocyanidin polymer comprises polymers of between about 2 to about 30 flavonoid units, between about 2 to about 15 flavonoid units, between about 2 to about 11 flavonoid units or an average of between about 7 to about 8 flavonoid units with a number average molecular weight of between about 2000 to about 3000 Da, or for example a molecular weight of between about 1100 daltons to about 2900 daltons; or for example a molecular weight of between about 1500 Da to about 3000 Da.

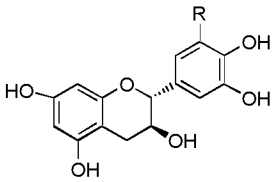
In one preferred embodiment, a proanthocyanidin polymer composition of the invention is Crofelemer. The structure of Crofelemer is shown below.



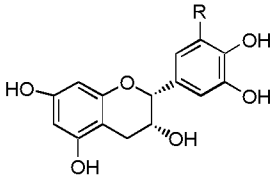
1

R= H (procyanidin) and/or R= OH (prodelphinidin)
Average n ~ 5 (7 units)

wherein, structure of monomeric units are



2 R= H, (+)-catechin
4 R= OH, (+)-gallocatechin



3 R= H, (-)-epicatechin
5 R= OH, (-)-epigallocatechin.

Analytical data

Assay and Taspine content

Assay is a method used to analyze or quantify a substance in a sample. An assay is an analysis done to determine the presence of a substance and the amount of that substance. Greater assay may promote the potency and clinical efficacy of that drug. Assay carried out by HPLC method by using C 18 column (150 X 4.6mm) and 0.1% trifluoroacetic acid in water. Tetrahydrofuran, methanol and acetonitrile were used as mobile phase with flow rate 1.5mL/minute. Measure the response for Crofelemer at UV 280 nm and for Taspine at UV 248 nm. Calculate the % Assay and taspine content against the standard.

$$\%\ \text{Assay (as such)} = \frac{\text{Area of Crofelemer in test solution at 280 nm}}{\text{Average area of Crofelemer at 280 nm}} \times \frac{\text{Weight of Crofelemer std.}}{20} \times \frac{5}{25} \times \frac{20}{\text{Sample Weight}} \times \frac{25}{5} \times \text{Potency of Crofelemer standard}$$

$$\text{Taspine (ppm)} = \frac{\text{Area of Taspine in test solution at 248 nm}}{\text{Average area of Taspine in reference solution at 248 nm}} \times \frac{\text{Weight of Taspine std.}}{100} \times \frac{1}{25} \times \frac{20}{\text{Sample Weight}} \times \frac{25}{5} \times \frac{\text{Potency of Taspine standard}}{100} \times 10^6$$

Related Substances

The term "impurities" refers to something that is impure or that makes something else impure. At present, the impurities are given various names some of the terms, such as related compounds, may tend to soft-pedal them. In the pharmaceutical world, an impurity is generally considered to be any other organic material besides the drug substance or active pharmaceutical ingredient (API) that arises out of synthesis. Most of the time, the inorganic contaminants are not given adequate consideration as impurities unless they are toxic, such as heavy metals or arsenic. Organic volatile impurities (OVI, which are generally made up of residual solvents as well as other organic volatile impurities used in the synthesis) are often considered virtual impurities. Interaction products produced during formulation processes and degradation products (frequently referred to colloquially as degradants in the pharmaceutical industry; the terms have been used interchangeably in this text) that can be produced prior to use by the patient are additional sources of impurities. It is important to recognize at this stage that any material that leads to a decrease in the purity value of the API should be considered an impurity. Therefore, for all intents and purposes, various contaminants mentioned here can be called impurities and should be labeled as such because they decrease the purity of API. Impurities are either naturally occurring or added during synthesis of a chemical or commercial product. During production, impurities may be purposely, accidentally, inevitably, or incidentally added into the substance. Greater impurities present in the drug may affect the potency and clinical efficacy of that drug. A measure of % impurity was carried out by HPLC method by using C18 column (150 X 4.6mm) and 0.1% trifluoroacetic acid in water. Tetrahydrofuran, methanol and acetonitrile were used as mobile phase with flow rate 1.5mL/minute. Measure the response at UV 280 nm.

$$\% \text{ Impurity at RRT of about 0.07} = \frac{\text{rea of Impurity at RRT of about 0.07 in test solution}}{\text{Average area of Crofelemer Std}} \times \frac{\text{Weight of Crofelemer standard}}{10} \times \frac{2}{10} \times \frac{1}{10} \times \frac{10}{\text{Weight of sample intest solution}} \times \frac{\text{Potency Crofelemer std.}}{100} \times 100$$

Polydispersity (D)

Polydispersity was measured using a HPLC system with quaternary gradient pumps, variable wavelength UV detector attached with data recorder and integrator software GPC Software option.

Column Jordi DVB, 500A°, 500 x 10mm using mobile phase DMF/ 50mM formic acid. Flow Rate 1.0 mL/minute at UV 280 nm perform injections of the calibration standard as a broad standard, using known values of M_n, M_w, M_z and M_p. The 1ST order (linear) was obtained. For system suitability, the r^2 value of the calibration curve was verified at 0.99 or greater,

Polymer Size Distribution was calculated using the following formula,

Polydispersity (D) or Heterogeneity (H)

$$D = H = \frac{M_w}{M_n}$$

Wherein, Mw is weight average molecular weight and Mn is number average molecular weight.

Diacetone content:

The diacetone content of Crofelemer is determined by Gas Chromatograph equipped with FID Detector and autosampler using HP-5 column (5% phenyl 95% methyl polysiloxane) dimension: 30mts*0.32mm*0.5um.

Water content (By KF method or technique):

Karl Fischer Instrument is an instrument used to determine the water content of a sample. Karl Fischer titration is a classic titration method in analytical chemistry that uses coulometric or volumetric titration to determine trace amounts of water in a sample.

Fill the titration vessel with the 15-20 mL of methanol. Press the start button on the instrument and wait until the display shows 'drift OK'. Change the parameters to 'KFT mode' and start. Add about 30.0 mg of water and enter the weight and press the start button. When the titration is complete, the display shows the burette reading. Note down the burette reading and calculate the K.F. Factor. (i.e. K.F. Factor = weight of water in mg / Burette reading). Change the instrument to 'KF mode'. Press the start button. Crush the sample to make it uniform. Transfer about 150 mg of the test sample into the titration vessel and enter the sample weight. Again press Enter. When the titration is complete, the display shows the burette reading. Note down the burette reading. Calculate the water content of the test sample using the following equation.

$$\text{Water Content (\%)} = \frac{\text{Burette reading x K.F. Factor}}{\text{Weight of sample in mg}} \times 100$$

Pharmaceutical Compositions

The pharmaceutical composition(s) described herein comprises Crofelemer and one or more pharmaceutically acceptable excipients, carriers, diluents or mixture thereof. Crofelemer as described herein may be associated with one or more pharmaceutically acceptable excipients, carriers, diluents or mixture thereof in the form of a capsule, sachet, paper or within any other container.

Examples of suitable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatin, lactose, terra alba, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethyl cellulose and polyvinylpyrrolidone.

The carrier or diluent may include a sustained release material, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

The pharmaceutical composition may also include one or more pharmaceutically acceptable auxiliary agents, wetting agents, emulsifying agents, suspending agents, preserving agents, salts for influencing osmetic pressure, buffers, sweetening agents, flavoring agents, colorants or any combination of the foregoing. The pharmaceutical composition of the patent application may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the subject by employing methods known in the art.

The pharmaceutical compositions of the present patent application may be prepared by conventional techniques, e.g., as described in *Remington: The Science and Practice of Pharmacy*, 20th Ed., 2003 (Lippincott Williams & Wilkins). For example, Crofelemer is mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, which may be in the form of an ampoule, capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material that acts as a vehicle, excipient or medium for the active compound. The active compound is adsorbed on a granular solid container, for example, in a sachet.

The pharmaceutical compositions may be in conventional forms, for example, capsules, tablets, aerosols, solutions, suspensions or products for topical application.

The route of administration may be any route which effectively transports Crofelemer to the appropriate or desired site of action. Suitable routes of administration include, but are not limited to, oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal, parenteral, rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic (such as with an ophthalmic solution) or topical (such as with a topical ointment). The oral route is preferred.

Solid oral formulations include, but are not limited to, tablets, capsules (soft or hard gelatin), dragees (containing the active ingredient in powder or pellet form), troches and lozenges. Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, cornstarch and/or potato starch. A syrup or elixir is used in cases where a sweetened vehicle is employed.

Liquid formulations include, but are not limited to, syrups, emulsions, soft gelatin and sterile injectable liquids, such as aqueous or non-aqueous liquid suspensions or solutions.

For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with Crofelemer dissolved in polyhydroxylated castor oil.

Suitable doses of the Crofelemer for use in treating the diseases and disorders described herein can be determined by those skilled in the relevant art. Therapeutic doses are generally identified through a dose ranging study in humans based on preliminary evidence derived from the animal studies. Doses must be sufficient to result in a desired therapeutic benefit without causing unwanted side effects. For example, the daily dosage of the Crofelemer can range from about 0.1 to about 30.0 mg/Kg. Mode of administration, dosage forms, suitable pharmaceutical excipients, diluents or carriers can also be well used and adjusted by those skilled in the art. All changes and modifications are envisioned within the scope of the present patent application.

In one embodiment, a pharmaceutical composition according to present invention, for use in the treatment of diarrhea, particularly secretory diarrhea. In one preferred embodiment, a pharmaceutical composition according to present invention, for the treatment of diarrhea associated with HIV/AIDS, irritable bowel syndrome, acute infection and pediatric diarrhea.

Experimental

The crude plant latex starting material used in the following examples is plant latex obtained from the bark of *Croton lechleri. Croton lechleri* trees were tapped and felled near the village of San Pablo de Cuyana on the Nanay River 100 kilometers from Iquitos, Peru. The latex was obtained over a period of 24 hours by scoring the trees.

The following abbreviations have been used herein:

HPLC: high performance liquid chromatography
FID: Flame ionization detector
RPM: revolutions per minute
mL: milliliters

g: gram

gm: gram

kg: kilogram

°C: degree centigrade

Example 1

Step A: Six volumes of water were added to crude plant latex (250 mL) comprising mud and the resultant mixture stirred at 40°C for 30 minutes and then filtered. The filtrate was kept undisturbed for 15 hours at 2 to 10°C. The solution was subjected to centrifugation for 10 minutes at 10°C and 3000 RPM, followed by decantation of the mother liquor (1750 mL).

Step B: 500 mL of purified water was added to the mother liquor. The mixture (2250 mL) was subjected to column purification using a 300 mL Sepharose column (1.2 volume of latex). The elution was carried out using 1500 mL of water followed by 30% aqueous acetone (1100 mL). The initial 300 mL eluent was discarded and then 750 mL of eluent (of dark colour) was taken and concentrated to yield 10.0 - 11.0 g of Crofelemer.

Example 2

Step-A: 250 mL crude plant latex solution comprising mud was lyophilized to obtain approximately 45-55 gm dried latex powder. 50 gm of freeze-dried latex were stirred with 2250 mL of purified water at 40°C for two hours. The solution was cooled to room temperature and filtered to obtain 2250 mL of mother liquor.

Step-B: The mass was subjected to column purification using a Sepharose column (300 mL, 6 volume of dried latex). The elution was carried out using 2000 mL of water followed by 30% aqueous acetone (1100 mL). The initial 300 mL eluent was discarded and then 750 mL of eluent (of dark colour) was taken and concentrated to yield 10.0 - 11.0 g of Crofelemer.

Example 3

Step A: Crude plant latex (35 kg) was mixed with water (240 L). The resultant mixture was stirred at 40°C for 60 minutes. The mixture was cooled to room temperature and

filtered through a sparkler filter to obtain a clear solution. The clear solution was subjected to ultrafiltration and concentrated upto 50 L.

Step B: 215 L of purified water was added to the concentrate mass. The resultant solution was subjected to column purification using a Sepharose column (36 L). Initially column was washed with 240 L purified water and the elution was carried out using 30% aqueous acetone (125 L).The initial 30-36 L eluent was discarded and then 90L of eluent (of dark colour) was taken and concentrated to yield 0.9 kg of Crofelemer.

Example 4

Step-A: 500 mL of crude plant latex solution comprising mud was lyophilized to obtain approximately 100-1 10 gm dried latex powder. 100 gm of freeze-dried latex was stirred with 4500 mL of purified water at 40°C for two hours. The solution was cooled to room temperature and filtered to obtain 5000 mL of mother liquor.

Step-B: The mother liquor was subjected to column purification using a Sepharose column (600 mL, 6 volumes of dried latex). Initially column was washed with 4 L purified water and the elution was carried out using 30% aqueous acetone (2200 mL). The initial 500 mL eluent was discarded and then 1500 mL of eluent (of dark colour) was taken and concentrated to yield 20.0 - 22.0 g of Crofelemer.

The analytical data for Example 1 to Example 4 are set forth in Table 1

Table 1

Analytical Data	Example 1	Example 2	Example 3	Example 4
Water content	14.44 %	-	11.12%	9.89%
Mn (Da) (number average molecular weight)	2097	2058	2411	2195
PD (polydispersity index)	1.03	1.03	1.004	1.03
Assay	90.17 %	92.72%	94.25%	93.61%
Taspine ppm	50	BDL	170.65	44
Gallocatechin	0.04 %	0.021%	0.029%	0.02%

Analytical Data	Example 1	Example 2	Example 3	Example 4
Epigallocatechin	0.07 %	0.062%	0.068%	0.04%
Catechin	0.01 %	BDL	BDL	BDL
Epicatechin	0.02 %	BDL	BDL	0.06%
Procyanadin-Bl	0.02 %	BDL	ND	BDL
Procyanadin-B2	BDL	BDL	ND	BDL
Impurities at RRT 0.07	0.01 %	BDL	BDL	BDL

* BDL= Below detection limit; * ND= Not detected

Reference Example- 1: Preparation of Crofelemer

Stage 1:

450 kg of latex was mixed with 925 L of water. The resultant mixture was mixed thoroughly at 2-8°C for an hour and allowed to settle at 2-8°C for 12 hours. The layers were separated. The upper layer was mixed with 200 L w-butanol. The mixture was stirred well. The layers were separated. The aqueous phase was extracted twice with 200 L ft-butanol each time. The aqueous phase was passed through a sparkler filter and then concentrated under reduced pressure to obtain solid.

Stage 2:

The 10 g of the solid material was mixed in 125 mL of purified water. The obtained solution was used in Reference Example 1.1 to 1.7.

Reference Example 1.1

The obtained solution of the material was applied into a chromatography system containing 118.0 mL CM-Sepharose column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.2

The obtained solution of the material was applied into a chromatography system containing 59 mL CM-Sepharose column (wherein, Bed Height = 6.5 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.3

The obtained solution of the material was applied into a chromatography system containing 41.3 mL CM-Sepharose column (wherein, Bed Height = 4.55 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.4

The obtained solution of the material was applied into a chromatography system containing 35.4 mL CM-Sepharose column (wherein, Bed Height = 3.9 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.5

The obtained solution of the material was applied into a chromatography system containing 29.5 mL CM-Sepharose column (wherein, Bed Height = 3.25 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.6

The obtained solution of the material was applied into a chromatography system containing 14.7 mL CM-Sepharose column (wherein, Bed Height = 1.62 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

Reference Example 1.7

The obtained solution of the material was applied into a chromatography system containing 7.2 mL CM-Sepharose column (wherein, Bed Height = 0.8 cm and Bed width = 3.4 cm) connected in series to a 118 mL LH-20 column (wherein, Bed Height = 13 cm and Bed width = 3.4 cm).

After the application of the feed to the top of the column, the columns were washed with 230 mL of purified water and 680 mL of 30% acetone. The columns were separated. The proanthocyanidin polymer composition was eluted from the LH-20 column with 450 mL of 45% acetone. The fractions containing desired proanthocyanidin polymer composition were concentrated to dryness and further dried.

The analytical data for Reference Example 1.1 to Reference Example 1.7 are set forth in Table 2

Table 2

Parameter	Reference Example 1.1	Reference Example 1.2	Reference Example 1.3	Reference Example 1.4	Reference Example 1.5	Reference Example 1.6	Reference Example 1.7
Feed Size (gm)	10	10	10	10	10	10	10
Column Diameter (cm)	3.4	3.4	3.4	3.4	3.4	3.4	3.4
CM-Sepharose bed height (cm)	13	6.5	4.55	3.9	3.25	1.62	0.8
Column Volume (mL)	118.0	59.0	41.3	35.4	29.5	14.7	7.2
Taspine (ppm)	150	150	131	1152	2073	17738	23601
Assay	89.06%	90.01%	100.48%	103.11%	95.06%	94.08%	89.10%

Reference Example 2: Preparation of Crofelemer (US 7.323.195)

Stage A:

450 kg of latex was mixed with 925 L of water. The resultant mixture was mixed thoroughly at 2-8°C for an hour and then allowed to settle at 2-8°C for 12 hours. The layers were separated. The upper layer was then mixed with 200 L w-butanol. The mixture was stirred well. The layers were separated. The aqueous phase was re-extracted twice with 200 L w-butanol each time. The aqueous phase was passed through a sparkler filter and then concentrated under reduced pressure.

Stage B:

The material from stage A (6 kg) was mixed in 75 L of purified water. The obtained material was applied into a chromatography system containing 35 L CM-Sepharose column connected in series to a 70 L LH-20 column. After the application of the feed to the top of the column, the columns were washed with 140 L of purified water

and 408 L of 30% acetone. The columns were separated. The proanthocyanidin polymer composition was eluted from the LH-20 column with 272 L of 45% acetone. The fractions containing desired proanthocyanidin polymer composition were concentrated to dryness and further dried.

The analytical data was collected for following batches. The analytical details for the composition of final proanthocyanidin polymer composition are given in the table 3.

Table 3

Batch No	Total impurities (%)	Taspine content (%)	Av. Mol. Wt (Dalton)
1	3.43	3.03	2496
2	4.43	3.66	2737
3	11.7	1.00	3183

Example 5

Preparation of Crofelemer

Stage A:

The drums containing latex were held at 2-8°C for not less than 48 hours before used. 450 kg of latex was mixed with 925 L of pre cooled water. The resultant solution was mixed thoroughly at 2-8°C for an hour and then allowed to settle at 2-8°C for 12 hours. The resulting precipitate was discarded. The supernatant was filtered through sparkler filter and then mixed with 200 L w-butanol. The mixture was stirred well. The layers were separated. The aqueous phase was re-extracted twice with 200 L w-butanol each time. The aqueous phase was concentrated to dryness under reduced pressure.

Stage B:

The material from stage A was dissolved in 75 L of purified water and the solution was filtered through sparkler filter. The operation was repeated to remove suspended particulates. The dissolved material was applied into a chromatography system containing 35 L CM-Sepharose Fast Flow column connected in series to a 70 L LH-20 column. After the application of the feed to the top of the column, the columns were

washed with 140 L of purified water and 408 L of 30 % acetone. The columns were separated. The proanthocyanidin polymer composition was eluted from the LH-20 column with 272 L of 45% acetone. The fractions were concentrated and dried to obtain Crofelemer.

The analytical details for a few representative batches for the Crofelemer are given in the following table 4.

The analytical data for a few representative batches of Example 5 are set forth in Table 4

Table 4

Batch No.	Assay (%)	Residual solvents (ppm)			Water content KF (%)	Taspine (%)	Total impurities (%)	Ave. mol. Wt. (da)
		acetone	*n*-butanol	Diacetone alcohol				
1	104.4	7	*3*	55	14.2	0	0.15	1935
2	99.9	16	*3*	37	14.2	0	0.07	1929
3	101.7	9	*3*	59	14.2	0	0.09	1943
4	100.3	3	*0*	36	10.9	0	0.08	1965
5	101	0	*0*	38	12.2	0	0.04	1997
6	103.7	4	*2*	55	12.3	0	0.04	2161
7	102.9	0	0	53	10.3	0	0.01	2012
8	104	8	0	56	12.2	0	0.01	1998
9	100.7	6	0	37	11.8	0	0.01	1993
10	101	0	0	23	11.4	0	0.01	2041

We claim

1. Crofelemer having a polydispersity index in the range of 0.9 to 1.2 obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring a mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

2. Crofelemer according to claim 1, obtained by the method described in claim 1.

3. Crofelemer wherein taspine is present in an amount of less than 500 ppm obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

4. Crofelemer according to claim 3, obtained by the method described in claim 3.

5. Crofelemer having an assay of greater than 85% obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

6. Crofelemer according to claim 5, obtained by the method described in claim 5.

7. Crofelemer having less than 0.15% of an impurity, preferably as measured at RRT 0.07, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

8. Crofelemer according to claim 7, obtained by the method described in claim 7.

9. Crofelemer having water content in the range of 7% to 17% (weight %), preferably when analyzed using KF technique, obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

10. Crofelemer according to claim 9, obtained by the method described in claim 9.

11. A method of producing Crofelemer comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using a column chromatography technique.

12. The method according to claim 11, wherein the process of isolating partially purified Crofelemer in step (A) comprising the steps of:

(a) stirring the mixture of crude plant latex or freeze-dried powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C;

(b) separating the liquid phase;

(c) optionally concentrating liquid phase to obtain solid, liquid or concentrated syrup; and

(d) optionally adding water or water miscible solvent(s) to the solid, liquid or concentrated syrup.

13. The method according to claim 12, wherein step (a) is performed at a temperature in the range of35°C to 45°C.

14. The method according to claim 12, wherein step (b) is performed by using filtration, sedimentation, centrifugation and/or decantation or combinations thereof.

15. The method according to claim 12, wherein step (b) is performed by using filtration.

16. The method according to claim 15, wherein filtration is performed with a filtering aid.

17. The method according to claim 12, wherein step (b) is performed by using sedimentation.

18. The method according to claim 17, wherein sedimentation is performed by keeping the plant latex mixture, solution or suspension undisturbed for time period of at least 1 hour with cooling at a temperature in the range of 5°C to 15°C.

19. The method according to claim 12, wherein step (b) is performed by using a centrifugation technique.

20. The method according to claim 19, wherein the centrifugation of the solution, suspension or mixture is performed at a temperature such as at 10°C using a speed in the range of from 100 to 10,000 revolution per minutes (RPM).

21. The method according to claim 20, wherein the centrifugation is performed at a speed in the range of from 2000 RPM to 4000 RPM, followed by decantation.

22. The method according to claim 12, wherein step (b) is performed using a combination of method(s) in the sequence of filtration, sedimentation followed by centrifugation and decantation.

23. The method according to claim 12, wherein step (c) is performed by using ultrafiltration and /or by using rotary evaporator or combination thereof.

24. The method according to claim 12, wherein step (c) is performed by ultrafiltration, followed by freeze drying, evaporation with heat, evaporation without heat, evaporation with vacuum, evaporation without vacuum, tray drying, spray drying, and combinations thereof to obtain solid, liquid or concentrated syrup.

25. The method according to claim 12, wherein in step (d) water is added to solid, liquid or concentrated syrup.

26. The method according to claim 11, wherein step (B) is performed by using ion exchange chromatography and/or size exclusion chromatography or combination thereof.

27. The method according to claim 11, wherein step (B) is performed by using ion exchange chromatography.

28. The method according to claim 27, wherein ion exchange chromatography is performed using a carboxy-methyl modified agarose.

29. The method according to claim 28, wherein the carboxy-methyl modified agarose is CM-Sepharose.

30. The method according to claim 11, wherein step (B) is performed by using combination of ion exchange chromatography and size exclusion chromatography.

31. The method according to claim 30, wherein step (B) is performed using a carboxy-methyl modified agarose and a hydroxypropylated cross-linked dextran.

32. The method according to claim 31, wherein the carboxy-methyl modified agarose is CM-Sepharose and the hydroxypropylated cross-linked dextran is Sephadex LH-20.

33. The method according to claim 11, wherein step (B) is performed using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1.

34. The method according to claim 11, wherein step (B) is performed using two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90:1.

35. The method according to claim 33 or 34, wherein the ratio of CM-Sepharose to the feed in a column is at least 4:1.

36. The method according to claim 33 or 34, wherein the ratio of CM-Sepharose to the feed in a column is at least 6:1.

37. The method according to claim 33 or 34, wherein the ratio of CM-Sepharose to the feed in a column is less than 9:1.

38. The method according to claim 33 or 34, wherein bed volume of CM-Sepharose column obtained after filling the CM-Sepharose suspension is at least 35 mL per 10 gram plant latex feed or substrate.

39. The method according to claim 38, wherein bed volume is 40.0 mL per 10 gm of the plant latex feed or substrate.

40. The method according to claim 38, wherein bed volume is 60.0 mL per 10 gm of the plant latex feed or substrate.

41. The method according to claim 33 or 34, wherein bed width (column inner diameter) of CM-Sepharose column obtained after filling the CM-Sepharose suspension is at least 3.2 cm per 10 gram plant latex feed or substrate.

42. The method according to claim 41, wherein bed width is 3.4 cm per 10 gm of the plant latex feed or substrate.

43. The method according to claim 33 or 34, wherein bed height (column length) of CM-Sepharose column obtained after filling the CM-Sepharose suspension is at least 4.2 cm per 10 gram plant latex feed or substrate.

44. The method according to claim 43, wherein bed height is 4.5 cm per 10 gm of the plant latex feed or substrate.

45. The method according to claim 34, wherein the ratio of Sephadex LH-20 to the feed in a column is at least 12:1.

46. The method according to claim 34, wherein bed volume of Sephadex LH-20 column obtained after filling the Sephadex LH-20 suspension is at least 110 mL per 10 gram plant latex feed or substrate.

47. The method according to claim 46, wherein bed volume is 118 mL per 10 gm of the plant latex feed or substrate.

48. The method according to claim 34, wherein bed width (column inner diameter) of Sephadex LH-20 column obtained after filling the Sephadex LH-20 suspension is at least 3.2 cm per 10 gram plant latex feed or substrate.

49. The method according to claim 48, wherein bed width is about 3.4 cm per 10 gm of the plant latex feed or substrate.

50. The method according to claim 34, wherein bed height (column length) of Sephadex LH-20 column obtained after filling the CM-Sepharose suspension is at least 8 cm per 10 gram plant latex feed or substrate.

51. The method according to claim 50, wherein bed height is 13 cm per 10 gm of the plant latex feed or substrate.

52. The method according to claim 11, wherein step (B) is performed by using an eluent(s) selected from water and water miscible solvent(s) and combination thereof.

53. The method according to claim 11, wherein step (B) is performed by using an eluent selected from water followed by an aqueous acetone solution.

54. The method according to claim 53, wherein 30% acetone in water is used as an eluent.

55. The method according to claim 11, wherein in step (B) fractions are collected with detectable absorbance in between 200-300 nM and additional UV absorption maxima from about 400-500 nM using UV spectroscopy.

56. The method according to claim 11, wherein in step (B) the concentration of an eluent is performed by using ultrafiltration and /or by using rotary evaporator or combination thereof.

57. The method according to claim 11, wherein Crofelemer comprising purity of greater than 98%.

58. The method according to claim 11, wherein the Crofelemer has an assay of greater than 85%.

59. The method according to claim 11 wherein the Crofelemer with a polydispersity index in the range of 0.9 to 1.20.

60. The method according to claim 11, wherein the Crofelemer has taspine present in an amount of less than 500 ppm, preferably as measured by HPLC.

61. The method according to claim 11, wherein the Crofelemer has less than 0.15% of an impurity, preferably as measured atRRT 0.07.

62. The method according to claim 11, wherein the Crofelemer has water content in the range of 7% to 17% (weight %), preferably as analyzed by KF method.

63. The method according to claim 11, wherein the Crofelemer is in amorphous form.

64. The method according to claim 11, wherein the Crofelemer has water content about 7 to about 17 percent by weight, and having a total organic compound impurity content less than about 1%, preferably as measured by HPLC.

65. Crofelemer having less than 0.15% of an impurity, preferably as measured at RRT 0.07.

66. Crofelemer having

a) polydispersity index in the range of 0.9 to 1.2;

b) taspine in an amount of less than 500 ppm;

c) an assay of greater than 85%;

d) less than 0.15% of an impurity, preferably as measured at RRT 0.07;

e) water content in the range of 7-17 % (wt %), preferably as analyzed by KF method;

f) amorphous form;

obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying the partially purified Crofelemer by using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1.

67. Cromfelmer according to claim 66, obtained by the process described in claim 66.

68. Amorphous Crofelemer having

a) polydispersity index in the range of 0.9 to 1.2;

b) taspine in an amount of less than 500 ppm;

c) an assay of greater than 85%;

d) less than 0.15% of an impurity, preferably measured at RRT 0.07;

e) water content in the range of 7% to 17% (weight %), preferably as analyzed by KF method;

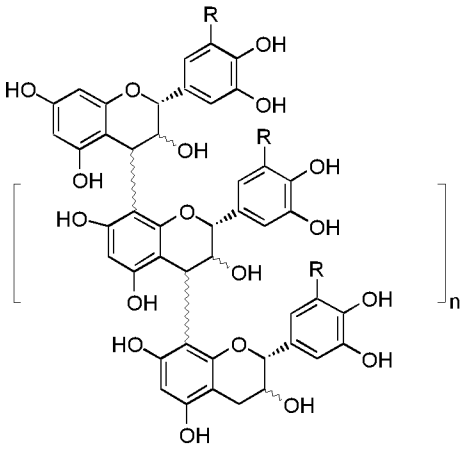
obtainable by a method comprising the steps of:

A) isolating partially purified Crofelemer by stirring mixture of crude plant latex or freeze-dried (lyophilized) powder of plant latex and water or water miscible solvent(s) at a temperature in the range of 15°C to 60°C; and

B) purifying partially purified Crofelemer by using single column of CM-Sepharose, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 or two set column of CM-Sepharose and Sephadex LH-20, wherein, the ratio of CM-Sepharose to the feed in a CM-Sepharose-column is in the range of about 3.5:1 to 11:1 and the ratio of Sephadex LH-20 to the feed in a Sephadex LH-20-column is in the range of about 8:1 to 90: 1.

69. Cromfelmer according to claim 68, obtained by the process described in claim 68.

70. Amorphous Crofelemer having formula



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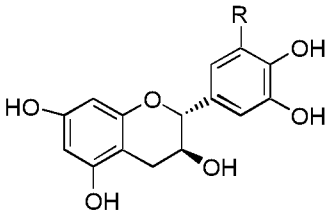
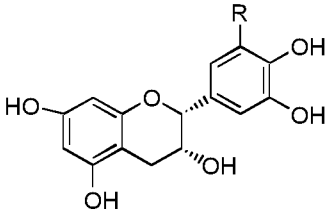
R= H (procyanidin) and/or R= OH (prodelphinidin)
Average n ~ 5 (7 units)

wherein, structure of monomeric units are



2 R= H, (+)-catechin
4 R= OH, (+)-gallocatechin



3 R= H, (-)-epicatechin
5 R= OH, (-)-epigallocatechin

71. Crofelemer containing less than 1000 ppm of any one of acetone, n-butanol, diacetone alcohol and combinations thereof.

72. Crofelemer according to claim 71, wherein diacetone content is less than 100 ppm.

73. Crofelemer according to claim 72, wherein acetone content is less than 150 ppm.

74. A pharmaceutical composition comprising Crofelemer according to any one of claims 1-10 and 65 to 73, or comprising Croflemer obtainable by a method according to any one of claims 11 to 64, and one or more pharmaceutically acceptable excipients, carriers, diluents or mixture thereof.

75. A pharmaceutical composition according to claim 74, for use in the treatment of diarrhea, particularly secretory diarrhea.

76. A pharmaceutical composition according to claim 74, for the treatment of diarrhea associated with HIV/AIDS, irritable bowel syndrome, acute infection and pediatric diarrhea.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/050658

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/47
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2011/024049 A2 (GLENMARK PHARMACEUTICALS LTD [IN] ; NAPO PHARMACEUTICALS INC [US] ; CHOW) 3 March 2011 (2011-03-03) claims ; examples	1-76
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-/- .

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

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"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
27 April 2012	07/05/2012

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

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/050658

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	FISCHER H ET AL: "A novel extract SB-300 from the stem bark latex of Croton lechleri inhibits CFTR-mediated chloride secretion in human colonic epithelial cells", JOURNAL OF ETHN0PHARMAC0L0GY, ELSEVIER SCIENTIFIC PUBLISHERS LTD, IE, vol. 93, no. 2-3, 1 August 2004 (2004-08-01), pages 351-357, XP008102355, ISSN: 0378-8741, D0I: 10. 1016/J .JEP.2004.04.005 [retrieved on 2004-06-01] page 352, column 1, paragraph 2 - page 352, column 2, paragraph 2 ----	1-76
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International application No
PCT/EP2012/050658

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