



(51) International Patent Classification:

A61K 36/48 (2006.01) *A61P 17/16* (2006.01)
A61K 8/97 (2006.01)

(21) International Application Number:

PCT/IB2008/050750

(22) International Filing Date:

29 February 2008 (29.02.2008)

(25) Filing Language:

English

(26) Publication Language:

English

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(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, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

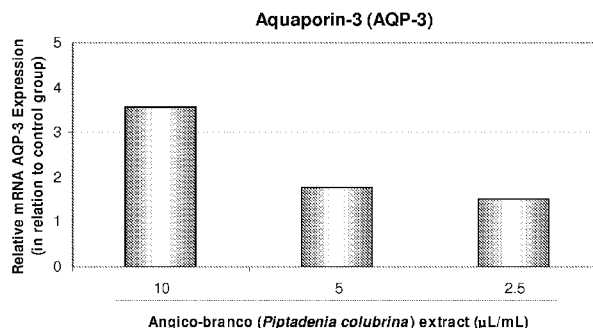
(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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

(54) **Title:** ANGICO-BRANCO (PIPTADENIA COLUBRINA) EXTRACTS FOR USE IN COSMETIC AND DERMATOLOGICAL FORMULATIONS

FIGURE 1



(57) **Abstract:** The present patent invention aims at the use of Angico-Branco (*Piptadenia colubrina*) extract as a skin barrier protective and moisturizing agent due to mechanisms involving the stimulation of aquaporin-3, fibronectin and envelope proteins - filaggrin and involucrin - expression, in formulations for cosmetics and dermatological products for the facial or body treatment of specific skin changes such as skin dryness, cracking, scaling, flaking or any disturbance involving skin barrier disruptions. The present patent invention is also related to the use of Angico-Branco (*Piptadenia colubrina*) extract in its crude form or extraction with organic (methanol, ethanol, isopropyl alcohol, ethylene glycol, butylene glycol, petroleum ether, ethylic ether, acetone, hexane) or for water extraction or with supercritical carbon dioxide (CO₂), pure or mixed with other extracts, oils and/or ingredients in preparations of cosmetic and dermatological products.



ANGICO-BRANCO (PIPTADENIA COLUBRINA) EXTRACTS FOR USE IN COSMETIC AND DERMATOLOGICAL FORMULATIONS

INVENTION FIELD

The present patent invention aims at the use of Angico-Branco (*Piptadenia colubrina*) extract as a skin barrier protective and moisturizing agent due to mechanisms involving the stimulation of aquaporin-3, fibronectin and envelope proteins – filaggrin and involucrin – expression, in formulations for cosmetics and dermatological products for the facial or body treatment of specific skin changes such as skin dryness, cracking, scaling, flaking or any disturbance involving skin barrier disruptions.

Additionally, the present patent invention is related to the use of Angico-Branco (*Piptadenia colubrina*) extract in its crude form or extraction with organic (methanol, ethanol, isopropyl alcohol, ethylene glycol, butylene glycol, petroleum ether, ethylic ether, acetone, hexane) or obtained through water extraction or with supercritical carbon dioxide (CO₂), pure or mixed with other extracts, oils and/or ingredients in preparations of cosmetic and dermatological products.

INVENTION BACKGROUND / PRIOR ART

With the claim for the use of Angico-Branco (*Piptadenia colubrina*) as a moisturizing agent due to mechanisms involving the stimulation of aquaporin-3, some products have been developed:

- Patent WO2007007255 - Personal care composition comprising at least one aquaporin-stimulating compound selected from the group consisting of xanthine, caffeine and other methylxanthines and plant extracts, like ginseng, promegranate and Ajuga turkestanica;
- Patent DE102006019794 - Use of glycerylglycosides and/or their derivatives for stimulating aquaporin expression in order to improve the water and/or moisture transport in the skin;

- Patent FR2874502 - Preparation of a concentrated extract of pomegranate able to amplify the expression of aquaporins;
- Patent JP2005343882 – A composition of the skin care preparation for external use comprises the extract or essential oil obtained from the extract or essential oil obtained from *Crocus sativus* in order to promote hyaluronic acid production and aquaporin synthesis;
- Patent EP1084700 - Antiperspirant contains an effective amount of aquaporin modulator.

With the claim of skin barrier protective due to mechanisms involving the stimulation of fibronectin and envelope proteins – filaggrin and involucrin, some products have been developed:

- Patent WO2006018454 – Skin compositions and identification of novel effects of molecules when incorporated into a skin compositions, specially systemic or topical compositions and their use in providing a variety of skin care benefits, as skin dryness prevention and treatment by boosting/maintaining involucrin levels;
- Patent WO2007032624 - Composition for external skin application having a skin-moisturizing effect, stimulating the expression of filaggrin and involucrin that are skin-moisturizing factors, and thus to provide excellent anti-drying and skin-moisturizing effects.
- Patent JP2007001914 – skin barrier function-improving agent which has an excellent skin cornification-normalizing action and improves skin dryness and chopped skin, enhancing the production of involucrin.
- Patent WO2007102957 – A skin care composition comprising a component which stimulates fibronectin synthesis, contributing for the reduction of wrinkles and improvement of skin elasticity and firmness;
- Patent WO2007100874 – A method for treating, ameliorating, and/or preventing the appearance of fine lines and wrinkles comprising topically applying to skin in an amount effective to increase fibronectin levels in skin cells.

BRIEF DESCRIPTION OF FIGURES

Figure 1 represents the relative mRNA expression of Aquaporin-3 (AQP-3) in human skin culture (co-culture) incubated with Angico-Branco (*Piptadenia colubrina*) extract (PCE) during 6 hours. Cultures were incubated with doses of 10, 5 and 2.5 $\mu\text{L/mL}$ of PCE during 6 hours. Total RNA was extracted from cells and AQP-3 relative expression was measured by Real Time-PCR analysis.

Figure 2 represents the relative mRNA expression of Fibronectin in human skin culture (co-culture) incubated with Angico-Branco (*Piptadenia colubrina*) extract (PCE) during 6 hours. Cultures were incubated with doses of 10, 5 and 2.5 $\mu\text{L/mL}$ of PCE during 6 hours. Total RNA was extracted from cells and Fibronectin relative expression was measured by Real Time-PCR analysis.

Figure 3 represents the relative mRNA expression of Filaggrin in human skin culture (co-culture) incubated with Angico-Branco (*Piptadenia colubrina*) extract (PCE) during 6 hours. Cultures were incubated with doses of 10, 5 and 2.5 $\mu\text{L/mL}$ of PCE during 6 hours. Total RNA was extracted from cells and Filaggrin relative expression was measured by Real Time-PCR analysis.

Figure 4 represents the relative mRNA expression of Involucrin in human skin culture (co-culture) incubated with Angico-Branco (*Piptadenia colubrina*) extract (PCE) during 6 hours. Cultures were incubated with doses of 10, 5 and 2.5 $\mu\text{L/mL}$ of PCE during 6 hours. Total RNA was extracted from cells and Involucrin relative expression was measured by Real Time-PCR analysis.

DETAILED DESCRIPTION OF THE INVENTION

The epidermis is itself a complex multiply layered membrane, yet varies in thickness from around 0.06 mm on the eyelids to around 0.8 mm on the load-bearing palms and soles of the feet. The epidermis contains no blood vessels and hence nutrients and waste products must diffuse across the dermo-epidermal layer in order to maintain tissue integrity. Likewise, molecules permeating across the epidermis must cross the dermo-epidermal layer in order to be cleared into the systemic circulation. The epidermis contains four histologically distinct layers which, from the inside to the outside, are the stratum germinativum, stratum spinosum, stratum granulosum and the stratum corneum.

The stratum corneum (SC) provides the major skin barrier to water loss and permeation of environmental substances, as well as contributing to mechanical protection. It is composed of multiples layers of non-viable, terminally differentiated corneocytes. The thickness of SC is highly variable, ranging from approximately 15 cells on the upper arm to hundreds on the palms and soles. In addition to this regional variation, the thickness of SC varies with sex, age and diseases. The barrier function is provided by a two-compartment system, often referred to as the “brick and mortar model”, of lipid-depleted, protein-rich corneocytes (bricks) surrounded by a continuous extracellular lipid matrix (mortar).

Typically, it takes 14 days for a daughter cell from the stratum basale to differentiate into a SC cell, and they are typically retained for a further 14 days prior to shedding. The barrier nature of the stratum corneum depends critically on its unique constituents; 75–80% is protein, 5–15% is lipid with 5–10% unidentified on a dry weight basis. The protein is located primarily within the keratinocytes and is predominantly alphakeratin (around 70%) with some beta-keratin (approximately 10%) and a proteinaceous cell envelope (around 5%). Enzymes and other proteins account for approximately 15% of the protein component.

Envelope proteins

The structure and functions of SC depend critically on the subcellular organization of epidermal proteins and lipids, as well as, on the water content. The cornified envelope is filled with bundles of keratin filaments aided by matrix proteins, including filaggrin, involucrin and loricrin. The cornified cell envelope (CE) is a specialized structure formed during terminal differentiation of stratified squamous epithelia and serves as a vital barrier for the tissue.

Filaggrin is a highly charged, cationic protein that aids aggregation and subsequent disulfide bonding of keratin filaments. It is derived from profilaggrin, a large (4400 kD) phosphorylated precursor expressed as keratohyalin granules in the granular layer of the epidermis. During the transition from the granular layer to the stratum corneum, profilaggrin is converted to filaggrin by site-specific proteolysis and dephosphorylation. In addition to profilaggrin processing to filaggrin, the transition from a granular cell to a corneocyte is characterized by the degradation of the nucleus and other organelles,

assembly of a cornified envelope, and reorganization of the keratin intermediate filament network into a two dimensional sheet. Filaggrin plays a critical role in the generation and maintenance of a flexible and hydrated stratum corneum and its hydrolysis is carefully regulated to generate free amino acids that form a major part of the natural moisturizing factors (NMF). The transition from a granular precursor, profilaggrin, to a diffusely distributed protein happens quickly at the granular to stratum corneum transition in response to an initiating signal which is not yet known. That profilaggrin is expressed as a precursor, rather than a mature protein, suggests that filaggrin expression must be regulated to prevent cytotoxic effects. Many inflammatory skin conditions are characterized by attenuation of the granular layer with concomitant parakeratosis, i.e. retained nuclei in the keratinocytes of the stratum corneum. While the signals that are disrupting terminal differentiation in these inflammatory conditions may be disparate, a common final theme is loss of the granular layer with subsequent incomplete terminal differentiation. In conditions where profilaggrin is decreased, such as atopic dermatitis, or essentially absent, as in ichthyosis vulgaris, the quality of the stratum corneum is compromised due to the inability of an NMF-depleted stratum corneum to remain hydrated under the desiccating action of the environment.

Corneocytes also have a characteristic envelope produced by cross-linking of precursor proteins such involucrin and loricrin, which imparts increasing rigidity to corneocytes during SC turnover. Involucrin was the first protein to be identified as a constituent of the cell envelope formed in epithelial cells. Involucrin is synthesized in the outer living layers of terminally differentiating keratinocytes of all stratified squamous epithelia. Human involucrin has been shown to be a preferred substrate of keratinocyte transglutaminase and a precursor of the cross-linked envelope. Reduced expression of human involucrin in transgenic mice results in abnormalities of the skin and hair.

Fibronectin

The adhesion of keratinocytes to each other, to the basal lamina and its interaction with the underlying dermis is another important feature involved in the regulation of epidermal homeostasis. In this respect, the multifunctional cell adhesion protein, fibronectin, is responsible for the process of tissue organization and skin re-

epithelialization. The glycoprotein fibronectin, like the proteoglycans and glycosaminoglycans, interact with other matrix components and with other matrix components and with other cells through specific receptors. As a consequence of their binding to other glycoproteins, collagen and elastic fibers, proteoglycans and to cells, glycoproteins are involved or mediate cell attachment (adhesion), migration, spreading, morphogenesis and differentiation. Fibronectin is an insoluble, filamentous glycoprotein synthesized in the skin by both epithelial and mesenchymal cells; it ensheathes collagen fiber bundles and the elastic network, it is associated with basal laminae and appears on the surfaces of cells, where it is bound to the cell through one of the multiple integrin receptors that mediate cell-matrix adhesion. Fibronectin also binds platelets to collagen; it is found in fibrin-fibrinogen complexes and plays a role in organizing the extracellular matrix. Fibronectin is involved in the wound healing process and so can be used as a therapeutic agent. It is also one of the few proteins for which production increases with age without any associated pathology. Fibronectin is also found in normal human saliva, which helps prevent colonization of the oral cavity by potentially pathogenic bacteria.

Aquaporin-3 (AQP-3)

Another important factor that plays a crucial role in cutaneous moisture pathways are the aquaglyceroporins-3/aquaporin-3 (AQP-3). The aquaporins (AQPs) are a family of membrane proteins that form water channels across cell membranes. Some aquaporins can also transport small solutes like glycerol or urea. Several hundred AQPs have been identified in various organisms including bacteria, yeast, insects, plants and animals. There are 13 aquaporins in mammals (named AQP-0 to AQP-12). AQP-1, 2, 4, 5 and 8 appear to function as selective water channels, while AQP-3, 7, 9 and 10 are also permeable to small solutes including glycerol. The tissue distribution, cellular localization, regulation, structure and function of mammalian AQPs have been extensively studied. However, the functional importance of AQPs is best understood in kidney, where AQP-1, 2, 3, 4 are involved in water reabsorption and urine concentration, whereas AQP-7 is involved in the transport of glycerol.

The epidermis is an epithelium which is permanently renewed: keratinocytes proliferate in the basal layer of the epidermis and they differentiate in the stratum spinosum

and stratum granulosum as they stratify and migrate towards skin surface. Finally, the stratum corneum (SC) is composed of terminally differentiated keratinocytes: corneocytes. The primary barrier of the epidermis against xenobiotics, loss of body fluids and water loss are the lipid matrix and corneocytes of the SC. The water content of skin is remarkably high in basal layers of the epidermis (~75% water) and drops sharply in the SC which contains only 10–15% water. Proper hydration of superficial SC, with highly organized lipid/water lamellar structures between corneocytes is important to maintain the permeability barrier of the skin, but lower SC is mostly unable to absorb water. The water content is crucial for the normal functioning of skin barrier, over all in the SC, which is especially mediated by the presence of membrane water channel proteins, aquaporin-3 (AQP-3). AQP-3 plays a crucial role in cutaneous moisture balance and stratum corneum hydration, determining the appearance and physical properties of the skin, and depends on several factors, including external humidity, skin structure, lipid/protein composition, barrier function, and concentration of water-retaining “humectants” such as free amino acids, ions and other small solutes. Studies have demonstrated that the non-expression of the water/glycerol transporting proteins AQP-3 in the several layers of human epidermis, leads to reduced SC hydration and skin elasticity, as well as, delayed barrier recovery. This water/glycerol channel exerts an important role preventing water loss. Besides, glycerol content correlates with lipid synthesis, which contributes to the maintenance of cutaneous function through the formation of a protective lipid film on skin surface. The basis of this film is sebum, which gets mixed with sweat and stratum corneum lipids, resulting in a water-retaining emulsified layer over the skin surface, which prevents trans-epidermic-water-loss (TEWL) and keeps the skin healthy and smooth.

Angico-branco (*Piptadenia colubrina*)

The leguminous tree Angico-branco (*Piptadenia colubrina* or *Anadenanthera colubrina*) is native to South American rain forests, growing at altitudes greater than 400 m. Its distribution is wide, varying in the north from Colombia and northern Brazilian States to the southern State of Parana, where it is known as Monjoleiro. Angico-branco belongs to the family Mimosaceae and is widely distributed in the Brazilian *caatinga*. It is particularly useful as a component in the second phase of reforestation projects and its wood can be

utilized in construction and carpentry, as well as in the production of charcoal. The bark of the tree is a possible source of tannin for the leather tanning industry. It is used in traditional medicine to treat respiratory problems, as an expectorant, and inflammations. The seeds of *Anadenanthera colubrina* are used to prepare *yopo*, a hallucinogenic inhalant used by the *curandeiros* of the *Piaroa* tribe inhabiting southeastern Venezuela. The use of *yopo* as a hallucinogenic among indigenous peoples of Latin America has been confirmed by archeological evidences.

Considering that skin hydration and epidermal barrier function have been active areas of investigation and that they are closely connected, we evaluated in the present study the effect of Angico-Branco (*Piptadenia colubrina*) in the gene expression of envelope proteins – filaggrin and involucrin, cell-adhesion molecule – fibronectin, and in the water-transporting protein aquaporin-3 (AQP-3).

DETAILED DESCRIPTION OF THE INVENTION

The present patent invention aims at the use of Angico-Branco (*Piptadenia colubrina*) extract as a skin barrier protective and moisturizing agent in formulations for cosmetics and dermatological products for the facial or body treatment of specific skin changes such as skin dryness, cracking, scaling, flaking or any disturbance involving skin barrier disruptions.

These effects are supported by mechanisms involving the stimulation of aquaporin-3, fibronectin and envelope proteins – filaggrin and involucrin – gene expression.

Angico-Branco (*Piptadenia colubrina*) extract can be used in its crude form or extraction with organic (methanol, ethanol, isopropyl alcohol, ethylene glycol, butylene glycol, petroleum ether, ethylic ether, acetone, hexane) or for water extraction or with supercritical carbon dioxide (CO₂), pure or mixed with other extracts, oils and/or ingredients in preparations of cosmetic and dermatological products.

These assertions are supported by *in vitro* efficacy tests, as explained below.

Evaluation of Aquaporin-3, Fibronectin, Filaggrin and Involucrin mRNA Expression by Real Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Human keratinocytes were incubated with Angico-Branco (*Piptadenia colubrina*) extract (10, 5 and 2.5 $\mu\text{L/mL}$) for a period of 6 hours. For RT-PCR analysis of aquaporin-3, fibronectin, filaggrin and involucrin, total RNA from keratinocytes after incubation was extracted and submitted to 1st strand cDNAs synthesis. Gene expression (cDNA) quantifications were performed by Real Time-PCR. Total RNA was extracted from cells and relative expression was measured by Real Time-PCR analysis.

RT-PCR analysis using aquaporin-3, fibronectin, filaggrin and involucrin primers were performed to study the effect on expression of the respective genes in human keratinocytes cells treated with Angico-Branco (*Piptadenia colubrina*) extract. The analyses demonstrated a stimulatory action of the extract in aquaporin-3, fibronectin, filaggrin and involucrin (figures 1, 2, 3 and 4, respectively) in all concentrations applied.

CONCLUSIONS:

Considering the following:

- The most superficial layer of skin is the stratum corneum (SC), which contains flattened dead epidermal cells (corneocytes) embedded in a lipid-rich matrix. The underlying epidermis consists of a viable keratinocyte multilayer that carries out the biosynthesis of lipids and proteins to be incorporated into the SC. The outermost layer of epidermis, constituted by corneocytes, provides a barrier that resists various insults from the external environment and prevents the loss of body fluid.
- The protective function of the mammalian epidermis depends largely on the presence of the chemically resistant envelope of the corneocytes. This envelope protects the cell from external injury, allows the attachment of keratin filaments and represents a permeability barrier to water and various substances. The multifunctional cell adhesion protein, fibronectin, together with envelope proteins filaggrin and involucrin, are responsible for the process of tissue organization and skin re-epithelialization, acting as a vital barrier for the tissue.
- Hydration of the SC depends on multiple factors, including its structure and composition, the external humidity, and the barrier and biosynthetic functions of the epidermal layer.

Epidermal keratinocytes contains aquaporin-3 (AQP3), a small integral membrane protein that functions as a facilitated transporter of water and glycerol, resulting in skin hydration and elasticity.

It could be concluded that:

- The Angico-Branco (*Piptadenia colubrina*) extract presented a stimulatory action of the aquaporin-3, fibronectin, filaggrin and involucrin in gene expression, according to all the results demonstrated above, attend to the claims requested.

CLAIMS

1. Use of Angico-Branco (*Piptadenia colubrina*) extract in dermatological and cosmetic formulations for the facial or body treatment of specific skin alterations.
2. The extract claimed in item 1 is characterized by being obtained from stems, leaves, branches, roots and fruits of Angico-Branco (*Piptadenia colubrina*) in its crude form or extraction with organic (methanol, ethanol, isopropyl alcohol, ethylene glycol, butylene glycol, petroleum ether, ethylic ether, acetone, hexane) or for water extraction or with supercritical carbon dioxide (CO₂), pure or mixed with other extracts, oils and/or ingredients in preparations of cosmetic and dermatological products.
3. The extract claimed in item 2 is characterized by the use, in any proportion of mixture and their parts separately, in formulations for cosmetics and dermatological products for the facial or body treatment of specific skin changes such as skin dryness, cracking, scaling, flaking or any disturbance involving skin barrier disruptions.
4. The extract claimed in item 3 is characterized by presenting as one of the action mechanisms, the stimulation of water channel aquaporin-3 gene expression, playing a crucial role in cutaneous moisture balance and stratum corneum hydration.
5. The extract claimed in item 4 is characterized by presenting as one of the action mechanisms, the stimulation of cell adhesion protein fibronectin gene expression, favouring the process of tissue organization and skin re-epithelialization.
6. The extract claimed in item 5 is characterized by presenting as one of the action mechanisms, the stimulation of cell envelope protein – fillagrin and involucrin – gene expression, which are essential for the maintenance of the cornified cell envelope working as a vital barrier for epidermal tissue.

FIGURE 1

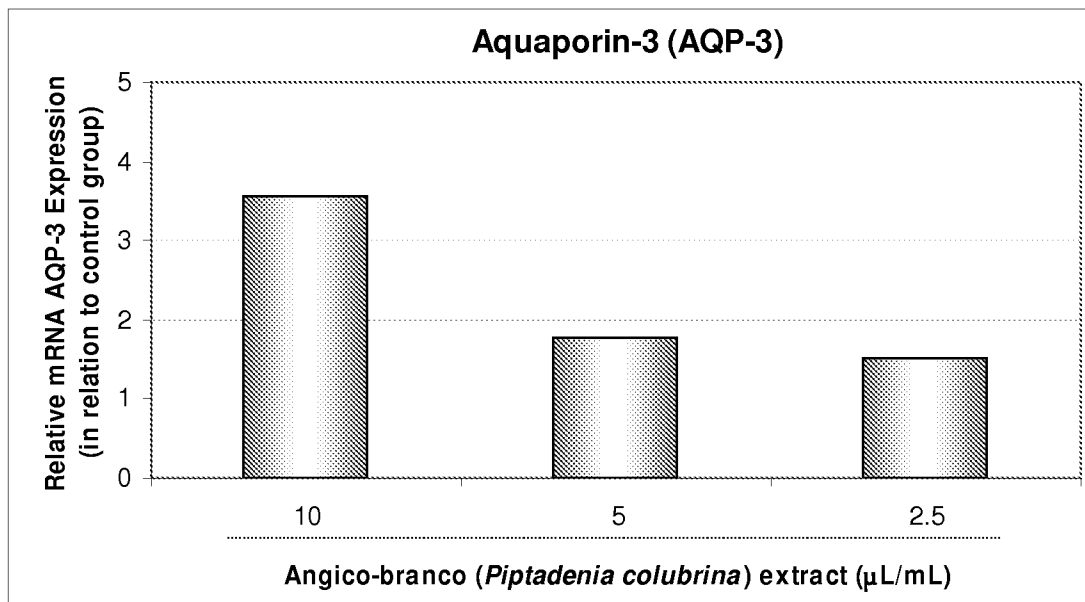


FIGURE 2

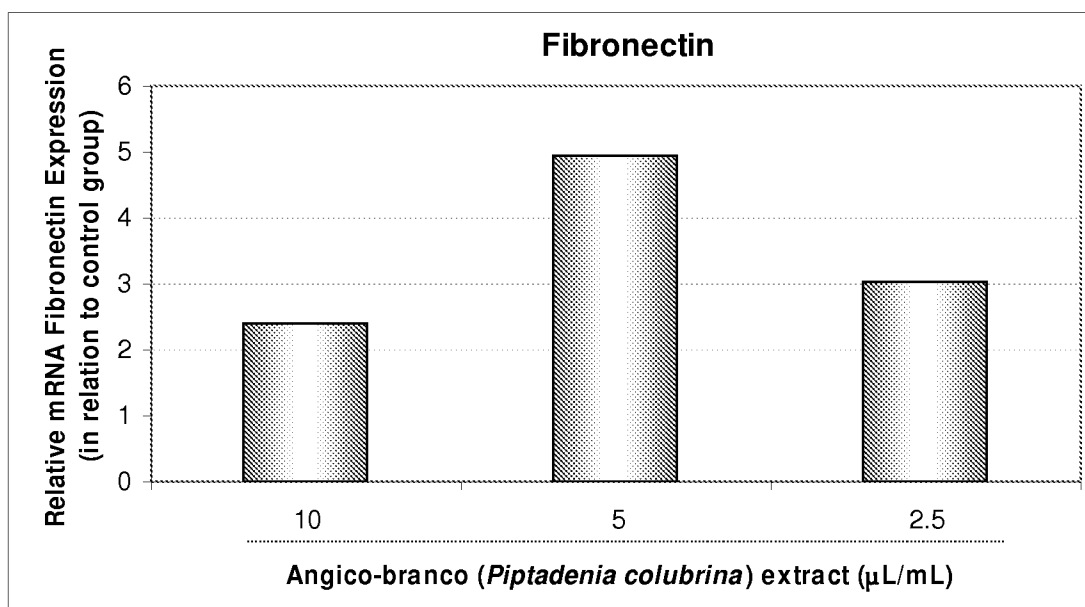


FIGURE 3

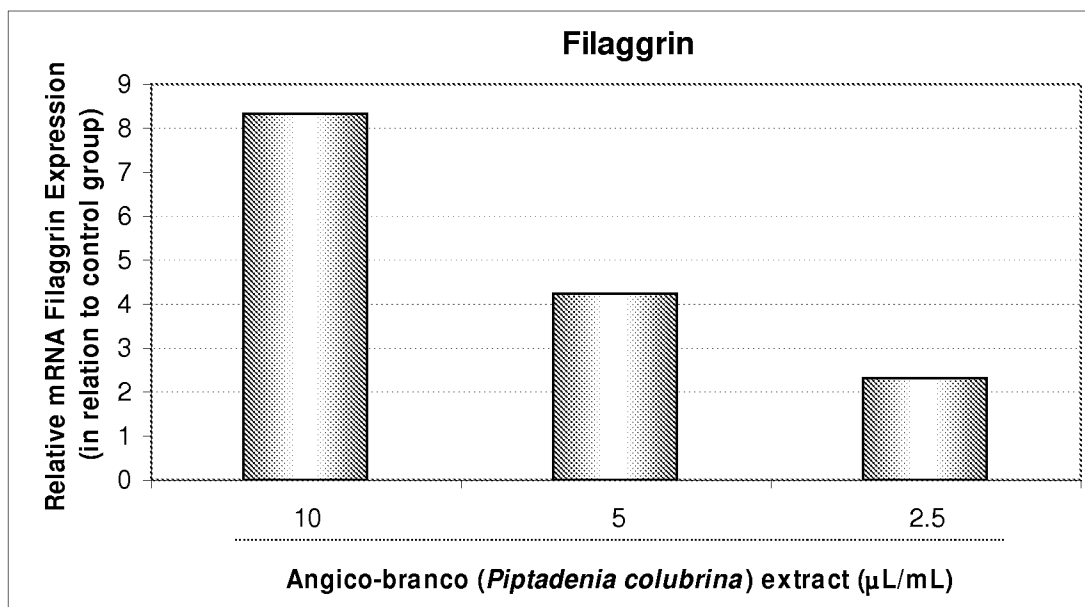
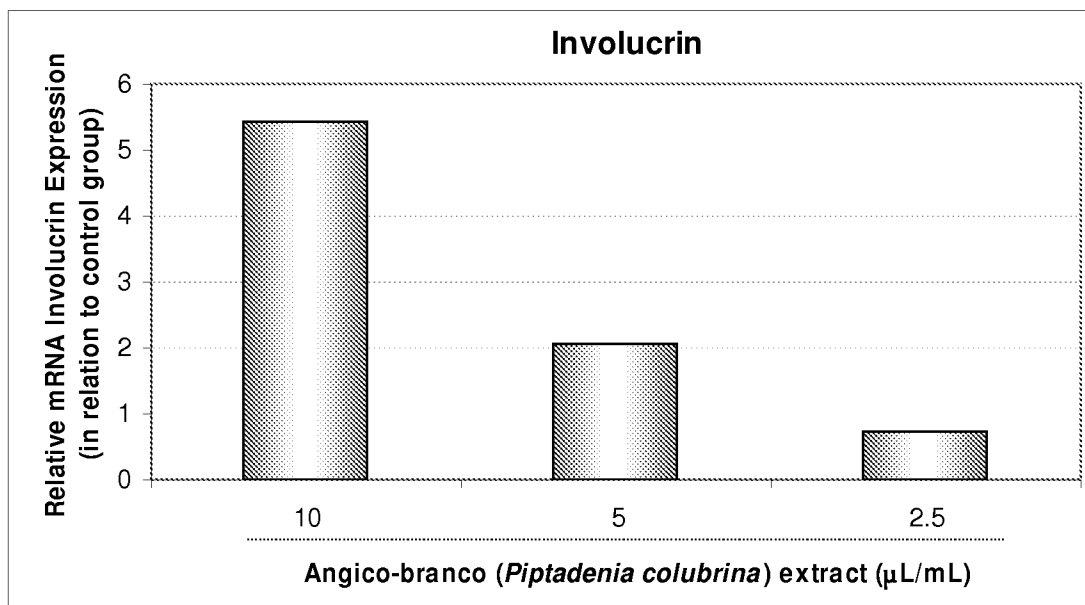


FIGURE 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2008/050750

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/48 A61K8/97 A61P17/16

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, MEDLINE, EMBASE, BIOSIS, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P,A	ANONYMOUS: "Anadenanthera macrocarpa" WIKIPEDIA, [Online] XP002489224 Retrieved from the Internet: URL: http://pt.wikipedia.org/wiki/Anadenanthera_macrocarpa [retrieved on 2008-07-22] the whole document	1-6

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

☒ See patent family annex.

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Date of the actual completion of the international search

23 July 2008

Date of mailing of the international search report

08/08/2008

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2008/050750

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2008/050750

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