More specifically, the anthocyamn is composed of cyanidin 3 glucoside, delphimdin 3 glucoside, and petumdin 3 glucoside.

The present invention relates to pharmaceutical compositions for wound healing, containing anthocyanin as an active ingredient, which is extracted from black soybeans, especially black soybean seed coats. More specifically, the anthocyanin is composed of cyanidin 3 glucoside, delphimdin 3 glucoside, and petumdin 3 glucoside.
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
Pharmaceutical composition for wound healing containing anthocyanin extracted from the black soybean seed coat

[Technical Field]

The present invention relates to pharmaceutical compositions for wound healing, containing anthocyanin as an active ingredient, which is extracted from black soybeans, especially black soybean seed coats. More specifically, the anthocyanin is composed of cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside.

[Background Art]

Anthocyanin is a water-soluble pigment glucoside, which exists in flowers, fruits, stems, leaves, and roots of plants. It is usually found in vacuoles of plants and it is a natural dye representing colors such as violet, red, and blue according to the acidity of cell sap, the chemical structure of dye compounds, and binding status with other metal ions. Anthocyanin is composed of aglycone and glycoside.

Currently the anthocyanin component is extracted from such plants as grapes, strawberries, olives, red cabbages, eggplants, roses, etc. and used as various materials. Yet, in fact, it is extracted and used almost as a co-pigment and often used with the compounds of other ingredients.
Anthocyanin, a natural vegetable dye, has recently been known to have some bioactivities. The well-known examples are anti-aging and antibacterial effect, anti-mutagenicity, cholesterol decrease, eyesight improvement, blood vessel protection, anti-ulcerative, and antioxidant effect. These bioactivities are basic for every kind of anthocyanin, but it was recently found that each anthocyanin has slightly different bioactivities.

It is known that anthocyanin is easily absorbed in intestines and it was also reported that its powerful antioxidant effect showed the protection ability for cells and tissues in animal models, in case of the ischemic infarction in the liver and heart and the acute inflammation in the lung (Takanori Tsuda et al., Archives of Biochemistry and Biophysics, 1999, 368 (2):361-366; Amorini A.M. et al, Free Radic. Res. 2003, 37 (4):453-460). Also, in Korean Laid-Open Patent Publication No. 2007-0026284, it is disclosed as a therapeutic agent that can protect the brain cell injury resulted from the stroke due to the complexity of the mechanism of brain injury.

A wound means an injured part of a body, including pathologically cut or damaged tissues in internal or external surface such as muscles, nervous tissues, bones, soft tissues,
internal organs, and vascular tissues. The examples are contusion, noncurative traumatic wound, tissue destruction by irradiation, abrasion, osteonecrosis, laceration, avulsion, penetrated wound, gun shot wound, incised wound, burn, frostbite, skin ulcer, xeroderma, palmoplantar keratoderma, rough and cracked skin, dermatitis, pain from dermatophytosis, operation wound, vascular disorder wound, cornea wound, decubitus ulcer, bedsore, diabetic and poor circulation related state such as diabetic skin erosion, chronic ulcer, stitched part after plastic surgery, spinal cord injury wound, gynaecological wound, chemical wound, and acne. The examples are not restricted by these and they also include some partial injuries.

Several therapeutic agents have been developed for wounds. Most of them have the granulation tissue proliferation action and the medication at the initial phase of burn may worse the symptoms. Also, regarding wound remedies containing iodine, there have been reports that they caused hypersensitiveness and delayed the cure due to cytotoxicity of iodine.

Yet, there have been no examples found that anthocyanin could be used to treat wounds.

On the other hand, there have been many studies on black soybeans since the Food and Drug Administration approved that
the soy protein helps prevent the coronary heart disease in 1998. It is known that black soybeans contain essential fatty acids, lecithin, fiber, saponin, etc., so that they have the effect to prevent and treat adult diseases such as hypertension, diabetes, arteriosclerosis, and obesity. The vegetable fiber contained in black soybeans is known to have the effect to prevent not only constipation but also colon cancer. Donguibogam, the Korean medical book written in the 17th century, describes black soybeans as "a drug that protects the five viscera, keeps intestines warm by helping twelve meridians, gets rid of boils, and treats radiating pain".

Also, anthocyanin, a pigment contained in black soybeans, has the anti-aging effect in case of a constant use as antioxidants. Isoflavone, the soy protein, contains saponin and phytosterol as well as genistin that have powerful anticancer activities, and helps calcium absorption into bones. Particularly, the seed coats of black soybeans contain an anticancer material called glycitein.

Anthocyanin is a water-soluble pigment glucoside and largely divided into six kinds of anthocyanidins depending on the number of the hydroxyl groups and the methylation of specific hydroxyl groups. It is also divided into hundreds of kinds depending on the sort of sugar, which is bonded with anthocyanidins, and the sort and bonding location of the acyl...
While conducting the study on wound treatment, the present inventors found to their surprises that anthocyanin extracted from black soybeans, especially from their seed coats was effective for treating wounds. It was also found that the damaged skin was rapidly cured or recovered when the agent containing the said purified anthocyanin as an active ingredient was applied on the wound, and the present invention was completed.

Therefore, the object of the present invention is to provide the pharmaceutical compositions containing anthocyanin extracted from black soybean seed coats to treat wounds.

The present invention relates to pharmaceutical compositions for wound healing, containing anthocyanin as an active ingredient, which is extracted from black soybeans, especially black soybean seed coats. More specifically, the anthocyanin is composed of cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside.
The purified anthocyanin of the present invention is composed of 65-80 weight% of cyanidin-3-glucoside, 15-25 weight% of delphinidin-3-glucoside, and 5-10 weight% of petunidin-3-glucoside.

The pharmaceutical compositions of the present invention, containing purified anthocyanin as an active ingredient with a pharmaceutically effective amount, can be formulated into various forms along with pharmaceutically permitted media. The formulation can be prepared according to the methods notified in the field.

The formulations whose active ingredients can be directly applied into local surface of skin include usual external preparations selected from the group consisting of ointment, gel, liquid (suspending agent, emulsion, lotion, etc.), cataplasm, tape, aerosol, powder, or dressing product, but they are not restricted by these examples. These formulations are described in the prescription paper, which is generally notified in pharmaceutical chemistry [Remington's Pharmaceutical Science, 15th Edition, 1975, Mack Publishing Company, Easton, Pennsylvania 18042 (Chapter 87: Blaug, Seymour)]. Also, the external preparation according to the present invention can be used after being adhered onto solid supports such as the release cover of the usual band aids. The
said dressing product provides damaged region with wet environment and helps the rapid recovery. There are closed and open dressing products. Most of them are the closed type such as film, foam, hydrocolloid, hydrogel, alginate, and collagen dressing products.

The pharmaceutically permitted media for the external preparation of the present invention are different depending on their formulations, but the examples are hydrocarbons such as Vaseline, liquid paraffin, and gelatinized hydrocarbon (also known as plastibase); animal or vegetable oils such as medium-chain triglyceride, lard, hard fat, and cacao butter; higher fatty acids such as cetanol, stearoyl alcohol, steric acid, isopropyl palmitate; fatty acids and their esters; water-soluble bases such as polyethylene glycol, 1,3-butyleneglycol, glycerol, gelatin, sucrose, and sugar alcohol; emulsifiers such as glycerin esters of fatty acids, polyoxyl stearate, and polyoxyethylene hydrogenated castor oil; pressure sensitive adhesives such as acrylate ester and sodium alginate; propellants such as liquefied petroleum gas and carbon dioxide; preservatives such as p-hydroxybenzoate ester. The external preparation of the present invention can be made using them according to the usual method. Also, other than these agents, stabilizers, perfumes, colorants, pH adjusters,
diluents, surfactants, preservatives, antioxidants, etc. may be compounded if necessary.

The external preparation of the present invention is applied on local wound regions according to the usual method.

In case of the ointment, cream, gel, and lotion, there could be compounds containing bases such as white Vaseline, yellow Vaseline, lanolin, white beeswax, cetyl alcohol, stearyl alcohol, stearic acid, hydrogenated oil, hydrocarbon gel, polyethylene glycol, liquid paraffin, squalene; solvents and solubilizing agents such as oleic acid, isopropyl myristate, glyceryl triisooctanoate, crotamiton, diethyl sebacate, diisopropyl adipate, hexyl laurate, fatty acid, fatty acid ester, aliphatic alcohol, and vegetable oil; antioxidants such as tocopherol derivatives, L-ascorbic acid, dibutyl hydroxy toluene, and butylated hydroxyanisol; antiseptics such as p-hydroxybenzoate; moisturizers such as glycerin, propylene glycol, sodium hyaluronate; surfactants such as polyoxyethylene derivative, glycerol ester of fatty acids, sucrose ester of fatty acids, sorbitan ester of fatty acids, propylene glycol ester of fatty acids, and lecithin; thickeners such as carboxy vinyl polymer, xanthan gum, carboxymethyl cellulose, sodium carboxymethyl cellulose, hydroxypropyl cellulose, and hydroxypropyl methyl cellulose;
propellants such as liquefied petroleum gas, liquefied carbon
dioxide, dimethylether, nitrogen, kerosene, and carbon
dioxide; stabilizers; preservatives; absorption enhancers; and
other suitable additives.

For cataplasms, there are compounds containing tackifiers
such as polyacrylic acid and polyacrylic acid copolymer;
crosslinking agents such as aluminium sulfate, aluminium
potassium sulfate, aluminium chloride, magnesium
aluminometasilicate, and dihydroxy aluminium acetate;
thickeners such as sodium polyacrylate, polyvinyl alcohol,
polyvinyl pyrrolidone, gelatin, sodium alginate, carboxymethyl
cellulose, sodium carboxymethyl cellulose, hydroxypropyl
cellulose, and hydroxypropyl methyl cellulose; polyhydric
alcohols such as glycerin, polyethylene glycol (macrogol),
propylene glycol, 1,3-butandiol; surfactants such as
polyoxyethylene derivatives; perfumes such as 1-menthol;
antiseptics such as p-hydroxybenzoate; purified water; and
other suitable additives.

For tapes, there are compounds containing pressure
sensitive adhesives such as styrene-isoprene-styrene block
copolymer and acryl resin; tackifiers such as alicyclic
saturated hydrocarbon type resin, rosin resin, and terpenoid
resin; softeners such as liquid rubber, liquid paraffin;
antioxidants such as dibutyl hydroxy toluene; polyhydric
alcohols such as propylene glycol; absorption enhancers such as oleic acid, surfactants such as polyoxyethylene derivatives; and other suitable additives. Also, aqueous tapes can be prepared with polymer that can contain water such as sodium polyacrylate or polyvinyl alcohol and a little amount of purified water.

For powder, there are compounds containing excipients such as potato starch, rice starch, corn starch, talc, zinc oxide, and other suitable additives.

For aerosols, there could be compounds, as in ointment, cream, gel, suspending agent, emulsion, liquid, lotion, and external powder, containing the followings: bases such as white Vaseline, yellow Vaseline, lanolin, white beeswax, cetyl alcohol, stearyl alcohol, stearic acid, hydrogenated oil, hydrocarbon gel, polyethylene glycol, liquid paraffin, squalene; solvents and solubilizing agents such as oleic acid, isopropyl myristate, diisopropyl adipate, diisopropyl sebacate, glycercyl triisooctanoate, crotamiton, diethyl sebacate, hexyl laurate, fatty acid, fatty acid ester, aliphatic alcohol, and vegetable oil; antioxidants such as tocopherol derivatives, L-ascorbic acid, dibutyl hydroxy toluene, and butylated hydroxyanisol; antiseptics such as p-hydroxybenzoate; moisturizers such as glycerin, propylene glycol, sodium hyaluronate; surfactants such as polyoxyethylene derivative,
glycerol ester of fatty acids, sucrose ester of fatty acids, sorbitan ester of fatty acids, propylene glycol of fatty acids, and lecithin; thickeners such as carboxyvinyl polymer, xanthan gum, carboxymethyl cellulose, sodium carboxymethyl cellulose, hydroxypropyl cellulose, and hydroxypropyl methyl cellulose; excipients such as potato starch, rice starch, corn starch, talc, and zinc oxide; propellants such as liquefied petroleum gas, liquefied carbon dioxide, dimethylether, nitrogen, kerosene, and carbon dioxide; buffers; correctives; suspending agents; emulsifiers; flavoring agents; preservatives, solubilizing agents; and other suitable additives.

The external preparation of the present invention is made using the usual preparation method such as mixing each component and necessary materials and used by the usual methods including a direct application on the injury or use by being coated or impregnated on clothes.

To prepare ointments, the ingredients are fat, fat oil, lanolin, beeswax, resin, plastic, glycols, higher alcohol, glycerin, water, emulsifier, suspending agent, and other suitable additives. Or using these as bases, the active ingredient is added and mixed to make the whole uniform. After the base components are melted with heat and mixed uniformly, additives such as absorption enhancers, antioxidants,
antiseptics, surfactants, purified water, etc. are added if necessary, and then micro powder of the active ingredient is also added and mixed to obtain the ointment or cream.

To prepare oleaginous ointments, for instance, ingredients are melted with heat and then mixed and half cooled. The active ingredient other than the bases is liquefied or micropowered to get mixed with some part of the bases. Then the rest of the bases are added and stirred for uniformity.

To prepare emulsion ointments and aqueous ointments, for instance, solid bases are melted in a water bath and kept at 75°C. Then, the aqueous bases are dissolved in water and those heated at the same or slightly higher temperature are added, and then mixed for uniformity.

To prepare cataplams, the active ingredient is mixed in advance with ointments having an aqueous polymer that has abundant water retention such as gelatin, carmellose sodium, methylcellulose, sodium polyacrylate, etc., and applied thinly on the support such as nonwoven fabrics. After the surface of the base is coated with plastic film such as polyethylene or polypropylene, it is cut for a desired size.

To prepare tapes, styrene-isoprene-styrene block copolymers or pressure sensitive adhesive such as acryl resins are added with tackifiers such as alicyclic saturated
hydrocarbon type resins, rosin resins, and terpenoid resins, softners such as liquid rubber and liquid paraffin, absorption enhancers, antioxidants, etc. This mixture is dissolved in organic solvents such as toluene and stirred or melted with heat. Into the stirred compound, the active ingredient on the liquid or powder state is added and mixed, which is then thinly applied on the release paper. In case of dissolved one, it is thinly applied on the paper and dried, and then layered on flexible supports such as polyurethane film, polyethylene film, polyvinyl chloride film, woven fabrics, and non woven fabrics and cut for a desired size.

To prepare lotions, the active ingredient, solvents, softners, suspending agents are added into aqueous liquid to make it uniform. The suspending lotion makes the active ingredient refined and glycerin or ethanol makes it easy to be wet in water. Then the suspending solution or lotion is slowly added to soften and make it uniform. For emulsion lotions, an oil-soluble agent and oil phase are put into one container and water phase into another, and then they are heated respectively. For the O/W type emulsion, oil phase is slowly injected into water phase. For the W/O type, on the contrary, water phase is slowly injected into oil phase and mixed until it obtains the complete emulsification and becomes uniformed liquid.
To prepare powder, the active ingredient and additives are uniformly dispersed into excipients such as potato starch, rice starch, corn starch, talc, zinc oxide, etc.

To prepare aerosols, after the solvent containing the active ingredient, ointment, cream, gel, suspending agent, emulsion, liquid, lotion, external preparations, etc. is made according to the said method, they are charged in a closed container with liquefied petroleum gas or compressed gas.

The skin wounds causing pain, which is the treatment object of the external preparation of the present invention, are temperature injuries such as burn, combustion, combustion ulceration, frostbite; traumas such as laceration, abrasion, incised wound, punctured wound, acne, and bite; blood and lymphatic vessel injuries such as Buerger's disease, lymphedema, and ulcer cruris; after-surgery wounds such as wounds from skin graft and sutured wounds; decubitus ulcer, pressure ulceration, diabetic ulcer and necrosis, postherpetic ulceration, drug-induced ulceration, stoma, irradiation injury, chemical injury and other skin wounds.

The pharmaceutically effective amount of anthocyanin, which is used for the pharmaceutical compositions of the present invention for wound treatment, means the amount of the active ingredient that shows the treatment effect by
normalizing abnormal cells and various cell activating materials in wounded parts. The effective dose may change depending on patients’ wound type, applied part, number of treatment, treatment period, formulation, patients’ state, assisting agent type, etc.

The amount of anthocyanin contained in the external preparation is different depending on formulations, but the effective dose is 0.01-50 weight% to the whole weight of the preparation, or desirably 0.1-10 weight%.

When the amount of anthocyanin is less than 0.01 weight%, wound treatment actions are not exhibited well enough and when it is more than 50 weight%, it is hard to prescribe the preparation. The number of dosage can be 3-4 times a day to once a week.

[Brief Description of Drawings]

Figure 1 is the picture showing full-thickness skin wounds of the control group and the experimental group in Experimental Example 1 (a) wound design of the control group and the experimental group; (b) wounds of the control group and the experimental group).

Figure 2 is the picture showing the result in vivo in Experimental Example 1 (21 days after the wounds).

Figure 3 is the graph displaying the comparison of wound
treatment between the control group and the experimental group in Experimental Example 1.

[Best Mode for Carrying Out the Invention]

Hereinafter, the present invention is described in more detail based on the following examples. But, these examples are not intended to limit the scope of the present invention.

[Example 1] Extraction and Purification of Anthocyanin

Using 200 g of black soybean seed coats (Glycine max (L) Merr.) cropped in the farmland of Gyeongsang National University at Jinju, it was extracted by methanol at 4 °C for 24 hours, which was repeated three times. The extraction was concentrated under reduced pressure and diluted until its volume reached 200ml. The solution containing anthocyanin was concentrated to be 100 ml and purified using Amberlite XAD-7 column and Spephadex LH-20. The purified anthocyanin was then composed of 72% of cyanidin-3-glucoside, 20% of cyanidin-3-glucoside, and 6% of petunidin-3-glucoside.

[Experimental Example 1] Comparing wound treatment effects according to anthocyanin and physiological saline solution

To find out the wound treatment effect of anthocyanin, the experiment was conducted as below. The hair on the back of
rats for experiments (Sprague-Dawley rat) was shaved and
the skin above the panniculus carnosus layer was eliminated to
make two symmetrical squares with a 2x2 cm size (as in Figure
1). The wound on the left, the control group, was coated with
the physiological saline solution and that on the right, the
experimental group, with the anthocyanin solution (the 'ANT'
solution hereafter), in which anthocyanin of Example 1 was
dissolved in distilled water to be 50mg/ml. On the wound (2cm x
2 cm), 0.1 ml of the ANT solution was coated at a time once a
day. The treatment progress was observed by measuring the
section of the wounds 7 days, 14 days, and 21 days after the
coating. Figure 2 and 3 show the result.

As shown in Figure 2, three weeks after the ANT solution
containing anthocyanin according to the present invention was
coated on the rats, it had better treatment effect than
physiological saline solution.

Also, Figure 3 shows the degree of treatment effect after
measuring the unhealed wound section. As shown in Figure 3, 7
days after the ANT solution was coated, the unhealed wound
section was 49.3 %, which means the half of the initial wound
section was cured. 14 days after the coating, 79% of the wound
section was healed and 21 days after the coating, 95% was
healed. On the contrary, when only physiological saline
solution was coated, it showed 32% of healed section after 7 days, 57% after 14, and 78% after 21.

[industrial Applicability]
The pharmaceutical compositions of the present invention for wound healing, containing anthocyanin as an active ingredient, which is extracted from black soybeans, especially black soybean seed coats, and composed of 65~80 weight% of cyanidin-3-glucoside, 15~25 weight% of delphinidin-3-glucoside, and 5~10 weight% of petunidin-3-glucoside, shows the excellent effect on wound treatment, so it can be used as a great wound healing agent.
[CLAIMS]

[Claim 1]
Pharmaceutical compositions for wound healing, containing anthocyanin as an active ingredient, which is extracted from black soybeans.

[Claim 2]
The pharmaceutical compositions for wound healing of claim 1, wherein said anthocyanin is extracted from black soybean seed coats.

[Claim 3]
The pharmaceutical compositions for wound healing of claim 2, wherein said anthocyanin is composed of cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside.

[Claim 4]
The pharmaceutical compositions for wound healing of claim 3, wherein said anthocyanin is composed of 65-80 weight% of cyanidin-3-glucoside, 15-25 weight% of delphinidin-3-glucoside, and 5-10 weight% of petunidin-3-glucoside.

[Claim 5]
The pharmaceutical compositions for wound healing of any one
of claims 1 to 4, wherein said compositions have the formulations selected from the group consisting of ointment, ointment adhesive, gel, liquid, cataplasm, tape, aerosol, powder, or dressing product.
Figures

Figure 1

(a) Control Group Experimental Group

(b) full-thickness wound
Figure 3

Unhealed Wound Section (%)

7 days  14 days  21 days

E.G. = experimental group, C.G. = control group
INTERNATIONAL SEARCH REPORT

PCT/KR2008/000513

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/7028(2006. 01)i

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)

IPC 8 A61K

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

Korean Utility models and applications for Utility models since 1975

Japanese Utility models and applications for Utility models since 1975

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

eKIPASS in KIPO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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* Special categories of cited documents

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

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

**T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search

08 MAY 2008 (08 05 2008)

Date of mailing of the international search report

08 MAY 2008 (08.05.2008)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
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LEE, JONG KOOG

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