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(54) Title: COMPOSITIONS CONTAINING BERBERINE OR ANALOGS THEREOF FOR TREATING ROSACEA OR RED FACE RELATED SKIN DISORDERS

(57) Abstract: The present invention discloses topical pharmaceutical formulations of berberine and its biologically equivalent analogues, such as palmatine and coptisine, for the treatment of rosacea and other red face-related skin disorders. The topical pharmaceutical formulations of this invention contain purified berberine as the primary active drug ingredient at concentrations higher than 0.1%. The invention also discloses methods of treating rosacea and other red face related skin disorders, such as steroid-induced rosacea-like dermatitis, comprising the administration of topical pharmaceutical formulations that contain berberine or its biologically equivalent analogues, such as palmatine.



WO 2011/000218 A1

## COMPOSITIONS CONTAINING BERBERINE OR ANALOGS THEREOF FOR TREATING ROSACEA OR RED FACE RELATED SKIN DISORDERS

### BACKGROUND OF THE INVENTION

#### 1. Rosacea and its major symptoms

Rosacea is a chronic skin disease that manifests redness and swelling, primarily on the face, especially on the central facial area. Other areas affected include the scalp, neck, ears, chest, back and the eyes. Rosacea is characterized by facial flushing, erythema, telangiectasia, and inflammatory episodes with papules and pustules and, in severe cases, rhinophyma. Comedones are notably absent<sup>1</sup>.

Patients with rosacea mostly have increased sensitivity of the facial skin and dry, flaking facial dermatitis, edema of the face, and persistent granulomatous papulonodules<sup>2</sup>. According to clinical and histopathologic features, the disease can be classified into 4 subtypes: (a) erythematotelangiectatic, (b) papulopustular, (c) phymatous, and (d) ocular, each with 3 grades of severity (mild, moderate, severe)<sup>3</sup>. The course of the disease is typically chronic, with recurrent remissions and relapses.

#### 2. Other red face related skin disorders

Rosacea is the most common red face skin disorder. Other red face related skin disorders, which share symptomatic similarities and probably pathological causes, include acne vulgaris, seborrheic dermatitis, photodermatitis and contact dermatitis. These red face related conditions may range from feelings of heat and sensitivity to flushing or burning with intense sensitivity<sup>4</sup>. Patients with rosacea and other red face related skin disorders often exhibit extreme sensitivity to environmental and topical factors<sup>5</sup>. Steroid-induced rosacealike dermatitis (or steroid rosacea) is a papular or pustular lesions with erythematous and edematous base with or without telangiectasia, which is caused by prolonged application of topical steroids to the face or as a rebound condition after discontinuation of topical steroids<sup>6,7</sup> (Chen AY Zirwas MJ, 2009; Lee DH, Li K, Suh DH 2008). EGFR inhibitors, such as cetuximab, erlotinib, gefitinib, cause acneiform dermatitis on face or other skin area, including papulopustular reaction, erythema, telangiectasias, and flushing in 30 to 90 % of patients and may also super-infected with bacteria, such as staphylococcus aureus<sup>8,9</sup> (Wollenberg A, Kroth J et al, 2010; Lacouture ME, Maitland ML et al, 2010).

#### 3. Pathogenesis of rosacea

The etiology of rosacea is not well understood. Various factors have been suggested to contribute to the development and manifestation of rosacea. None of them, however, has been definitely confirmed<sup>1</sup>.

##### 3.1. Genetic contribution

Earlier studies have indicated genetic predisposition to flushing, the earliest manifestation of facial rosacea<sup>10</sup>. Additionally, glutathione S-transferase MU-1 (GSTM1) and glutathione S-transferase theta 1 (GSTT1) null genotype has been reported to be associated with an increased risk of rosacea<sup>11</sup>.

### 3.2. Inflammation and innate immune system

As rosacea progresses, inflammatory lesions become evident. Unlike acne vulgaris, inflammatory rosacea is not a bacterial disease of the pilosebaceous unit. Comedones are usually not present, and only normal bacterial flora is identified in skin samples taken from rosacea patients<sup>12</sup>. The inflammatory stage of rosacea can be regarded as a form of chronic sterile cellulitis<sup>13</sup>. While the presence of microorganisms has been examined as a potential contributing factor to rosacea, results have been inconclusive<sup>1</sup>. *Demodex folliculorum* mites are considered as commensal and do not play a significant pathogenic role in rosacea, although an inflammatory reaction to the mites may aggravate symptoms<sup>14</sup>.

Yamasaki *et al* found an abnormally high level of cathelicidins by histopathological staining in skin lesions from patients with rosacea. Human epidermal keratinocytes stimulated by cathelicidin peptides were found to increase the release of IL-8. Injection of cathelicidin peptides into the skin of mice caused inflammatory changes with increased neutrophil infiltration and microvessels characteristic of the skin disorder of rosacea in humans<sup>15</sup>. Cathelicidins possibly have dual roles in immunity because it can both kill microorganisms and stimulate host inflammatory responses such as inducing IL-8 release<sup>16</sup>. Other inflammatory cytokines found to be increased in rosacea include IL-1alpha and transforming growth factor beta-2<sup>17,18</sup>.

### 3.3. Vascular mediators

Inflammatory mediators may be responsible for the vasodilation seen in rosacea patients. For example, substance P, histamine, serotonin, bradykinin, or prostaglandins have been suggested<sup>19</sup>. Smith *et al* has reported an increased expression of vascular endothelial growth factor and its receptors in rosacea<sup>20</sup>.

## 4. Current management of rosacea

A number of antibiotics, such as tetracycline and doxycycline have been used in treating rosacea. It has been suggested that such antibiotics render anti-inflammatory rather than antimicrobial effects. However, other anti-inflammatory agents are not effective in treating rosacea. Immunosuppressive agents such as corticosteroids often worsen the inflammatory condition of rosacea<sup>1</sup>.

Topical metronidazole and certain systemic antibiotics are often used as first-line therapy for rosacea. Oral tetracycline, doxycycline, and minocycline are commonly used for treating rosacea. The efficacy of oral antibiotics is probably due more to anti-inflammatory rather than to antibiotic effects<sup>21</sup>. Azelaic acid 15% gel was approved by FDA of USA in 2002 for the topical treatment of mild to moderate rosacea<sup>22</sup>. Other traditional topical agents that have been used in a "off label" fashion include clindamycin, sulfacetamide and sulfur, but their mechanism is not well understood.

## 5. The use of berberine in non-skin disorders

Berberine (Natural Yellow 18, 6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo (5,6-a) quinolinizinium) is an isoquinoline alkaloid present in herb plants, such as coptis (*Coptidis rhizome*), phellodenron, *Scutellaria baicalensis*, *Mahonia aquifolium* and berberis<sup>23</sup>. Berberine

and its derivatives have been found to have antimicrobial and antimalarial activities. It can act against various kinds of pathogens such as fungi, saccharomycete, parasite, bacterium and virus<sup>24</sup>. Berberine has been found to have other potential benefits. For example, it may have potential to treat high blood cholesterol, cardiovascular disease, diabetes, and tumor<sup>25</sup>.

Berberine also has anti-inflammatory function, yet the exact mechanism is unknown. Recently, some researcher reported that the anti-inflammatory mechanism of berberine is mediated through cyclooxygenase-2 (COX-2) pathway, since COX-2 plays a key role in the synthesis of prostaglandins, which is elevated in inflammation<sup>26</sup>. Berberine is used as an ingredient in some eye drop solution or eye ointment for the treatment of trachoma<sup>27</sup>.

## **6. The use of berberine in skin disorders**

US patent #6440465 pertains to topical skin formulations of glucosamine in an emollient base which contains berberine for the treatment of psoriasis<sup>28</sup>. Patent application #20050158404 pertains to a nutritional product, dietary supplement or pharmaceutical composition which contains vitamin A, vitamin E, selenium, vitamin B6, zinc, chromium, and a herbal source of berberine for the treatment of acne in oral administration<sup>29</sup>. US patent #6974799 relates to topical compositions comprising a tripeptide (N-palmitoyl-Gly-His-Lys) and a tetrapeptide (N-palmitoyl-Gly-Gln-Pro-Arg) for the treatment of visible signs of aging including wrinkles, stretch marks, dark circles<sup>30</sup>. The formulation may contain additional ingredients, including berberine. In these inventions, berberine is included as one of the many ingredients and its concentration is not specified.

Patent application #20040146539 relates to topical neutraceutical compositions with body slimming and tone-firming anti-aging benefits that may be used to treat skin aging, skin wrinkle, skin exfoliating, acne, rosacea and other skin problems<sup>31</sup>. The composition of this invention includes antimicrobial agents selected from several agents including berberine. In these neutraceutical compositions, berberine is included as one of the many ingredients and its concentration is not specified. There has been a 10% Mahonia aquifolium cream (Relieva<sup>TM</sup>, Apollo Pharmaceutical Canada Inc) containing 0.1% berberine for the treatment of psoriasis<sup>32</sup>.

The therapeutic effect of berberine in treating rosacea and other red face-related skin disorder is unknown. Until now, there is no direct evidence suggesting that berberine can improve the symptoms of rosacea.

## **DESCRIPTION OF THE INVENTION**

There is a need for an effective therapy for the treatment of rosacea and other related skin disorders with minimal side effects. The present invention pertains to topical pharmaceutical formulations that are effective and safe in treating rosacea and other red face related skin disorders, such as acne, seborrheic dermatitis, contact dermatitis and photodermatitis. This invention recognizes the deficiency in currently available topical pharmaceutical formulations or experimented formulations that contain berberine as a component, and improves over this deficiency.

There are lines of evidence that indicate that berberine is a drug active ingredient in animal studies and human clinical trials of berberine, either with purified berberine or formulations containing berberine herbal extract. In many disease indications, such as in the treatment of bacterial and fungal infections and cardiovascular diseases, statistically significant efficacy

results of berberine have sometimes been obtained. In the trials on psoriasis with formulations containing berberine-rich extract, efficacious results were also obtained, although the efficacy of berberine in psoriasis has not been accepted. These results suggest that berberine can act on molecular targets and cause modifications in certain molecular pathways and cellular functions, such as described in the background section in this patent application.

It has been clearly shown in the pharmacological studies of numerous pharmaceutical compounds that a pharmaceutically active compound must be present in the body or affected tissues above certain threshold concentrations for the drug to achieve meaningful biological and pharmacological effects and hence therapeutic effects in the treated subject. In herbal medicinal preparations that contain the extracts of one or multiple plant(s), many active drug ingredients are present. In most treatments using herbal preparations either in an oral or topical route, the individual drug ingredients are present at sub-threshold concentrations in the body or affected tissues of a treated subject. However, several compounds from the same or different plants may act on the same molecular target or several compounds from the same plant or different plants may act on different molecular targets in the same biological pathway. As a result, the various compounds acted concertedly to cause a meaningful biological and pharmacological effect and hence therapeutic effect.

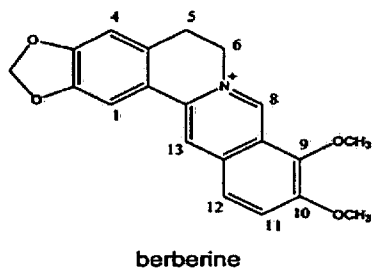
When a herbal pharmaceutical preparation fails to cause a therapeutic effect in a treated subject, it is likely that an otherwise pharmacologically active compound contained therein is present at too low a concentration in the treated subject and that the compound by itself or in combination with compounds in the preparation fail to cause a meaningful biological and pharmacological effect. In fact, many important drug compounds (single chemical entities) have been identified and isolated from plants that are used in herbal preparations. With these pure compounds, therapeutic efficacy often exceed that is achievable with the herbal preparations that contain the compounds.

Topical herbal pharmaceutical formulations that include berberine-rich plant extracts have been used for centuries in the treatment of various ailments, including a variety of skin disorders, such as psoriasis, acne, eczema, etc. These topical herbal preparations have achieved variable results. In some of those preparations, berberine –containing extract consists of about 10% of the various components used to constitute the formulation. It has been estimated that the berberine compound in those topical total preparations accounts approximately 0.1 % (w/w) of the finished formulations<sup>32</sup>.

Based on the above rationale, we have investigated *in vitro* the effects of berberine at various concentrations on biological pathways that may be involved in the pathogenesis of rosacea. Based on those results and the rationale described above, we have developed chemically defined topical pharmaceutical formulations that contain berberine at defined percentages that are higher than the concentrations of berberine in traditional herbal berberine-containing pharmaceutical formulations. We then tested those formulations on affected skin area on patients with rosacea. Our findings indicate that topical pharmaceutical formulations containing berberine above 0.1% (w/w) can achieve efficacious and tolerable results in treating rosacea and related sensitive red face disorders.

### **Analogues of berberine**

The structure of berberine (5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo (5,6-a) quinolinizinium) is shown below:



Several protoberberine alkaloids can be prepared with variable biological activity similar to berberine, such as: jatrorrhizine, palmatine, coptisine, 9-demethylberberine, 9-demethylpalmatine, 13-hydroxyberberine, berberrubine, palmatrubine, 9-O-ethylberberrubine, 9-O-ethyl-13-ethylberberrubine, 13-methyldihydroberberine N-methyl salt, tetrahydroprotoberberines, and their N-methyl salts, 13-Hexylberberine, 13-hexylpalmatine and 9-lauroylberberrubine chloride<sup>33,34</sup>.

Palmatine is present in plants of various families, most notably in the rhizomes of *Fibrarurea Tinctoria Lour*. Palmatine is an isoquinoline alkaloid and formulations containing palmitine have been broadly used in China for the treatment of gynecological inflammation, bacillary dysentery, enteritis, respiratory tract infection, urinary infection. Additionally, palmatine has the function of anti-arrhythmia, antisepticise, bacteriostasis, and anti-viral activities. Palmatine can be also used as a compound in anti-tumor drug screening<sup>35</sup>. There has been a palmatine-containing pharmaceutical as topical hair growth inhibitor (Keramene, Divine Skin Solutions D S Laboratories Keramene Body Hair Minimizer).

Coptisine is an alkaloid found in Chinese goldthread (*Coptis chinensis*). It is used in Chinese herbal medicine along with the related compound berberine for treating digestive disorders caused by bacterial infections. Coptisine also exhibits some significant inhibition on tumor growth. Coptisine has been shown *in vitro* to be cytotoxic on human tumor colon cell line<sup>36</sup>, human hepatoma and leukaemia cell lines<sup>37</sup>.

In our studies, we have also investigated *in vitro* and *in vivo* the effects of palmatine, and coptisine at various concentrations on biological pathways that may be involved in the pathogenesis of rosacea. Based on those results and the rationale described above, we have also developed chemically defined topical pharmaceutical formulations that contain palmatine or coptisine at defined concentrations. These formulations could achieve efficacious and tolerable results in treating rosacea and related sensitive red face disorders.

#### **Example 1: Effects of berberine on inhibiting cathelicidin peptides-induced cytokine secretion by human keratinocytes (*in vitro* assay)**

For our *in vitro* study, berberine (Sigma, St. Louis, MO, USA) was dissolved in water, methanol, ethanol or dimethyl sulfoxide (DMSO). Normal human keratinocytes (Invitrogen, CA, USA) were grown in EpiLife medium (Invitrogen, CA, USA) supplemented with 0.06 mM Ca<sup>+2</sup>, 1% EpiLife defined growth supplement, and 1% penicillin/streptomycin (Invitrogen, CA, USA). Cells were grown at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. The human keratinocytes were cultured to confluence and treated with synthetic cathelicidin peptides (LL-37) (6.4 μM) for 16 h to induce inflammatory response similar to that observed in rosacea. Some of the cathelicidin-treated keratinocyte cultures were co-incubated with berberine of concentrations from 1.25 μg/ml to 12.5 μg/ml. The keratinocytes cultures treated with cathrlicidin or cathrlicidin with 1% ethanol and without berberine were used as negative controls. Supernatants were collected and placed in a sterile 96-well plate for ELISA of interleukin-8

(IL-8), interleukin-1 alpha (IL-1 alpha), and venous epithelial cell growth factor (VEGF) in accordance with the manufacturer's instructions (R&D Systems, MN, USA).

The result showed that cathelicidin can induce IL-8, IL-1 alpha and VEGF release from cultured human keratinocytes. The inhibitory effect of berberine on the release of IL-8 (Figure 1A), IL-1 alpha (Figure 1B) and VEGF (Figure 1C) was examined by adding different concentrations (0~12.5 µg/ml) of berberine in the culture medium. There was 31.4%, 24.9 % and 29.1% decrease of the release of IL-8, IL-1 alpha and VEGF respectively, when cathelicidin-stimulated keratinocytes treated with 1.25 µg/ml berberine comparing to cathelicidin peptide-treated with 1% ethanol control ( $P < 0.05$ ). These results showed that berberine can significant inhibit cathelicidin induced inflammatory response in a dose-dependent manner, especially when the concentration of berberine was larger than 6.25 µg/ml, indicating that berberine has anti-inflammatory activity against cathelicidin-induced release of cytokines, which were related to rosacea.

### **Example 2: Preparation of topical pharmaceutical formulations containing purified berberine and palmatine at defined percentages**

Based on the rationale described above, the topical berberine-containing pharmaceutical formulations of this invention have one key feature: it contains purified berberine at defined percentages that are higher than can be obtained in previous formulations using extracts of berberine-rich plants. The ranges of concentrations were subjected to tests in animal model studies and human clinical studies.

For our studies on animal models and human patients, purified berberine was dissolved in 100% ethanol, and then water was added to reach a desired concentration of berberine in the final solution. In the gel formulation, for example, 0.1% or 0.2% berberine was prepared in 10% ethanol. The solution or gel formulation were capped and stored at 4°C until use. The results of our studies in animal models and human patients with rosacea indicate that the concentration of berberine in the formulation should be 0.1% or higher, in order to achieve consistently satisfactory results. These concentrations are higher than previously prepared topical berberine-containing formulations using berberine-rich plant extract.

Experiments are on going to prepare formulations in the form of an ointment, gel, cream, lotion, or spray, which are more suitable for use for clinicians and patients. In the topical pharmaceutical formulations of our invention, berberine or a biologically equivalent analog of berberine (e.g. palmatine and coptisine) is the only or primary active drug compound. The purified palmatine used for our studies is dissolved in 100% water, and then diluted to reach in the final solution or gel formulation with defined palmatine concentrations, for example, 0.02%, 0.1%, or 0.2% of palmitine.

However, improved or modified formulations may include additional ingredients for increased solubility of berberine or its analogue, emulsification, lubrication, antibiotic activity, or hydration.

One preferred embodiment of our invention to increase the solubility of berberine or a biological equivalent analog of berberine is to add glycerol into the formulation. One embodiment of our invention to increase the antibiotic activity of the formulation is to add plant extract that has been shown to have antibiotic activity. One embodiment to enhance the hydration property of the topical formulation of our invention is to add hyaluronic acid.

**Example 3: The effects of the topical pharmaceutical preparation of this invention on a mouse model of rosacea**

The animal model of rosacea: the animal model of rosacea was adopted from previous reported<sup>18</sup>. Briefly, BALB/c and C57BL/6 mice, shaved 24 h before treatments, were injected subcutaneously on the back with 40  $\mu$ l of cathelicidin peptide (320  $\mu$ M) twice a day. Forty-eight hours after the initial injection (four injections in total), erythema and edema were observed on the injected site mimicking the clinical features of rosacea.

In our experiments, cathelicidin-injected mice were treated with or without topical berberine twice a day to observe the effect of berberine on reducing inflammation. The results showed that mice given subcutaneous injections of cathelicidin peptides induced erythema and vascular dilatation in the skin, which resembled clinical features of rosacea after 48 h. The cathelicidin-injected mice were then divided into 2 groups, which were treated with berberine (n=3) or not treated with berberine (n=3; as controls), respectively, for a subsequent 2 days. The topical formulation containing 0.1% berberine was applied on the cathelicidin-induced lesions twice a day. The erythematous or inflammatory lesions lasted for more than 7 days in the control group. At the 4<sup>th</sup> day, erythema and vascular dilatation were significantly reduced in the berberine treated group comparing to controls. These results indicate that topical berberine can reduce the inflammatory reaction induced by cathelicidin *in vivo*.

**Example 4: A human clinical study investigating the efficacy of the topical pharmaceutical formulation of this invention on patients with rosacea**

**Method:** an open-label clinical study was carried out to determine the efficacy of the topical berberine formulations of this invention for the treatment of rosacea and related skin disorders. Patients included in this study were diagnosed by dermatologists to have clinically defined rosacea. All patients were given 0.1% berberine gel twice a day for 6 weeks. At the time points of treatment initiation, and 2-weeks and 6-weeks after treatment, the patients were evaluated for their rosacea symptoms. The patients were not allowed to use other medications, including antibiotics, for their skin conditions. Only oral antihistamines were allowed for relief of pruritus symptoms.

To evaluate the efficacy of treatment, the standard grading system for rosacea developed by the National Rosacea Society Expert Committee on the Classification and Staging of Rosacea was used<sup>3</sup>. Additionally, the investigator's global assessment (IGA) and overall erythema severity of the patients were scored at week 0, week 2, and week 6 of berberine therapy. The IGA was expressed according to a 7-point scoring system with a range of 0 (clear) to 6 (severe). The severity of overall facial erythema and telangiectasia, respectively, was graded as 'none', 'mild', 'moderate', or 'severe' with scores from 0 to 3. The grading system used to assess overall facial erythema severity was described previously<sup>38</sup>.

**Results:** a total of 20 patients with rosacea (18 females and 2 males) were enrolled in this study. The mean age of the study population was 43.3 (19-85) years. The mean duration of rosacea prior to berberine treatment was 4 (1-24) years. Among the 20 patients with rosacea, 13 cases were of erythematotelangiectatic type (65%), 7 cases papulopustular type (35%), and 5 cases (25%) phymatous type.

According to the 7-point score system, IGA score of rosacea at baseline (initiation of treatment) was  $4.1 \pm 1.3$ . This score decreased to  $2.6 \pm 0.9$  at week 2, then  $1.6 \pm 0.8$  at week 6. The difference of IGA scores between week 0, week 2, and week 6 was statistically significant (W2



vs W0: paired t test  $P < 0.0001$ ; W6 vs W0: paired t test  $P < 0.0001$ ). At the beginning of treatment, the majority of patients (95%) had grading from mild to moderate (3) to severe (6). By the end of treatment, 19 of the 20 patients (95%) had a mild (2) to clear (0) rating.

The overall erythema severity evaluated by the investigator was  $2.35 \pm 0.6$  at the beginning of treatment,  $1.5 \pm 0.5$  at week 2, and  $0.95 \pm 0.4$  at week 6. The improvement at week 2 or week 6 was statistically significant (W2 vs W0: paired t test  $P < 0.0001$ ; W6 vs W0: paired t test  $P < 0.0001$ ). At the beginning of treatment, the majority of patients (95%) had erythema rating from moderate (2) to severe (3). By the end of treatment, 19 of the 20 patients (95%) had a mild (1) to none (0) erythema rating.

**Safety and tolerability:** There was no serious adverse event during the study. Only 2 cases (10%) had transient itchy/stinging sensation in the area of topical medication, but were tolerable without discontinuation of study.

**Example 5: Topical berberine was effective for the treatment of steroid-induced rosacea-like dermatitis and EGFR inhibitors-induced acneiform dermatitis**

We also studied the 0.1% berberine gel on 10 patients with steroid-induced rosacea-like dermatitis and 5 patients with EGFR inhibitors-induced acneiform dermatitis, using a regimen of twice a day for 6 weeks. All 15 patients showed symptomatic improvement and tolerated the topical treatment as observed with rosacea.

**Example 6: Palmitine showed efficacy for the treatment of rosacea or red face disorders:**

We also studied topical formulation containing palmitine at 0.02% (w/w) on 10 patients with rosacea and related red face disorders. All 10 patients showed symptomatic improvement and tolerated the topical treatment as observed with berberine.

**Conclusion made from the examples:**

*In vitro* culture studies have demonstrated that berberine exhibits anti-inflammatory effects by inhibiting cathelicidin-induced IL-8, IL-1 alpha and VEGF production by human keratinocytes. Since inflammation is involved in the pathogenesis of rosacea and related skin disorders, the anti-inflammatory effects of berberine may account for its clinically beneficial effect in rosacea and related inflammatory skin disorders.

The results of our clinical studies have shown that the topical pharmaceutical formulations of this invention containing purified berberine at concentrations higher than 0.1% or palmitine at concentrations higher than 0.02% can be efficacious, safe and well tolerable for the treatment of rosacea and red skin related disorders, such as acne, contact dermatitis, seborrheic dermatitis and photodermatitis, steroid-induced rosacealike dermatitis, and EGFR inhibitors induced acneiform dermatitis..

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1. Berberine inhibited cathelicidin peptide (LL-37)-induced IL-8, IL-1alpha and VEGF release from human keratinocytes. Keratinocytes were stimulated by cathelicidin peptide (LL-37), and the release of IL-8 (Figure 1A), IL-1 alpha (Figure 1B) and VEGF (Figure 1C) by the keratinocytes was evaluated by ELISA assay.

Figure 2. A. Investigator's global assessment scores at the beginning of berberine treatment and at 2 weeks and 6 weeks of treatment. B. Overall erythema severity scores at the beginning

of topical berberine treatment and at week 2 and week 6 of treatment.

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## CLAIMS

## WHAT IS CLAIMED:

1. A topical pharmaceutical composition for treating red face related skin disorders, comprising at least 0.02% w/w of berberine or a biologically equivalent analogue thereof.
2. The topical pharmaceutical composition of claim 1, wherein berberine or the biologically equivalent analogue thereof is the primary pharmaceutically active component.
3. The topical pharmaceutical composition of claim 1, wherein berberine or the biologically equivalent analogue thereof is the only pharmaceutically active component.
4. The topical pharmaceutical composition of claim 1, wherein the red face related skin disorder is selected from the group consisting of rosacea, acne vulgaris, seborrheic dermatitis, photodermatitis, contact dermatitis, steroid-induced rosacealike dermatitis, and EGFR inhibitors- induced acneiform dermatitis.
5. The topical pharmaceutical composition of claim 4, wherein the red face related skin disorder is rosacea.
6. The topical pharmaceutical composition of claim 1, wherein the concentration of berberine or the biologically equivalent analogue thereof is between about 0.1% and about 2% w/w.
7. The topical pharmaceutical composition of claim 1, further comprising a solvent selected from the group consisting of water, methanol, ethanol, and dimethyl sulfoxide (DMSO).
8. The topical pharmaceutical composition of claim 1, further comprising one or more components selected from the group consisting of solubilizers, emulsifiers, lubricants, and hydratants.
9. The topical pharmaceutical composition of claim 8, further comprising glycerol, hyaluronic acid and/or a plant extract.
10. The topical pharmaceutical composition of claim 1, further comprising one or more agents for treating acne and/or rosacea.
11. The topical pharmaceutical composition of claim 10, wherein the agent for treating acne and/or rosacea is an antibiotic or antibacterial agent.
12. The topical pharmaceutical composition of claim 10, wherein the agent for treating acne and/or rosacea is selected from the group consisting of azelaic acid, retinoids, metronidazole, benzoyl peroxide, sodium sulfacetamide, and sulfur.
13. The topical pharmaceutical composition of claim 1, in the form of a solution, lotion, gel, cream, ointment, or spray.

14. The topical pharmaceutical composition of claim 1, wherein the biologically equivalent analogue of berberine is selected from the group consisting of jatrorrhizine, palmatine, coptisine, 9-demethylberberine, 9-demethylpalmatine, 13-hydroxyberberine, berberrubine, palmatrubine, 9-O-ethylberberrubine, 9-O-ethyl-13-ethylberberrubine, 13-methyldihydroberberine N-methyl salt, tetrahydroprotoberberines and N-methyl salts thereof, 13-Hexylberberine, 13-hexylpalmatine, and 9-lauroylberberrubine chloride.
15. The topical pharmaceutical composition of claim 14, wherein the biologically equivalent analogue of berberine is palmatine or coptisine.
16. A method for treating red face related skin disorders, comprising topically applying to affected skin a therapeutically effective amount of a pharmaceutical composition comprising berberine or a biologically equivalent analogue thereof.
17. The method of claim 16, wherein the red face related skin disorder is selected from the group consisting of rosacea, acne vulgaris, seborrheic dermatitis, photodermatitis, contact dermatitis, steroid-induced rosacealike dermatitis, and EGFR inhibitors induced acneiform dermatitis.
18. The method of claim 17, wherein the red face related skin disorder is rosacea.
19. The method of claim 16, wherein the pharmaceutical composition comprises at least 0.02% w/w of berberine or a biologically equivalent analogue thereof.
20. The method of claim 19, wherein the pharmaceutical composition comprises about 0.1% to about 2% w/w of berberine or a biologically equivalent analogue thereof.
21. The method of claim 16, wherein the biologically equivalent analogue of berberine in the pharmaceutical composition is selected from the group consisting of jatrorrhizine, palmatine, coptisine, 9-demethylberberine, 9-demethylpalmatine, 13-hydroxyberberine, berberrubine, palmatrubine, 9-O-ethylberberrubine, 9-O-ethyl-13-ethylberberrubine, 13-methyldihydroberberine N-methyl salt, tetrahydroprotoberberines and N-methyl salts thereof, 13-Hexylberberine, 13-hexylpalmatine, and 9-lauroylberberrubine chloride.
22. The method of claim 21, wherein the biologically equivalent analogue of berberine is palmatine or coptisine.

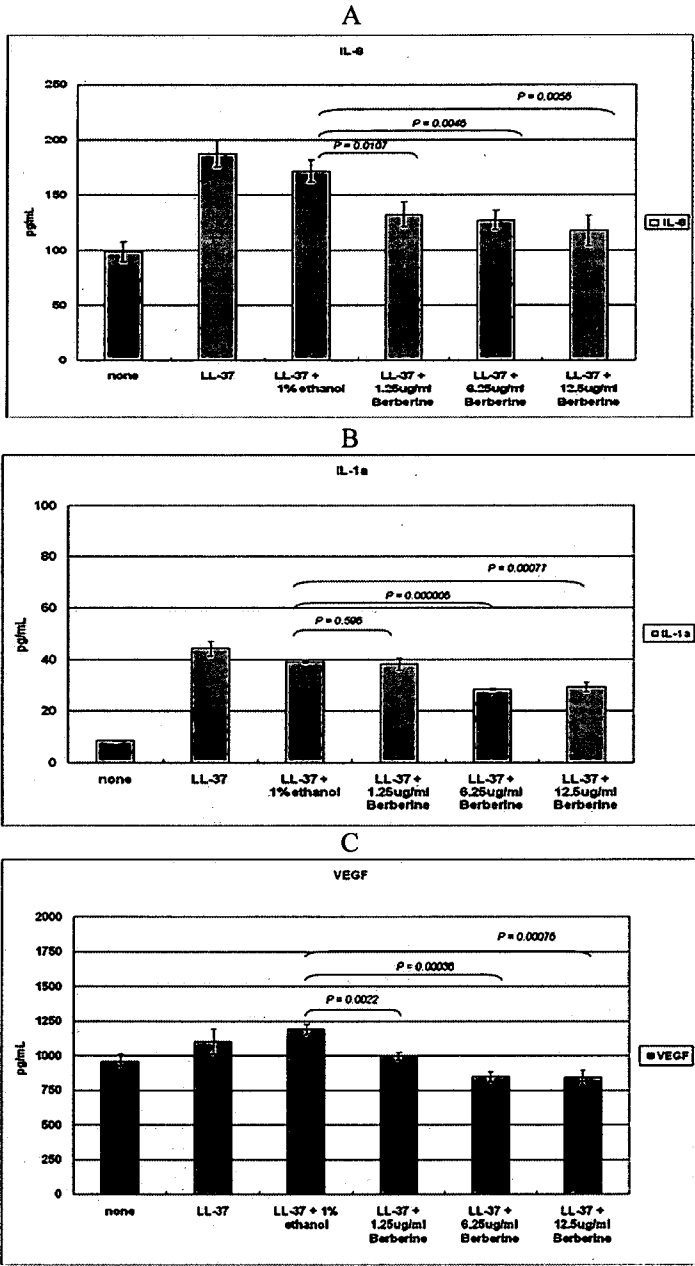


Figure1

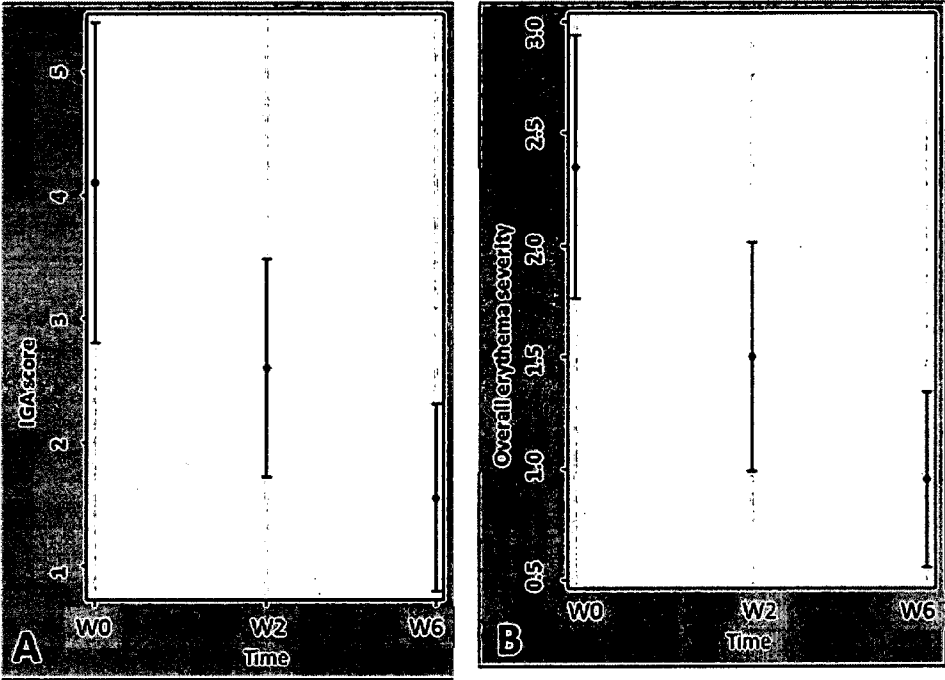


Figure 2



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/000983

## A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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: 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 practicable, search terms used)

DWPI, EPODOC, CNPAT, Chinese patent full text search system, Chinese medical patent search system, CNKI, CA: berberine, jatrorrhizine, palmatine, coptisine, +berberine?, +palmatine?, +berberrubine?, rosacea, red face, dermatitis, acne, water, methanol, ethanol, dimethyl sulfoxide, solubilizer?, emulsifier?, lubricant?, hydrantant?, glycerol, hyaluronic acid, plant, antibiotic, antibacterial, azelaic acid, retinoid?, metronidazole, benzoyl peroxide, sodium sulfacetamide, sulfur, topical,

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 1923199 A (INST OF DERMATOLOGY CHINESE AC(CN)) 07 March 2007 (07.03.2007) see claims 1-2, example 2	1-9,13-16,19-22
X	CN 1182788 A (XIONG Guangming (CN)) 27 May 1998 (27.05.1998) see claim 1, description page 2 lines 6-7, 9	1,4-5,9-13,16-17
X	KR 20030082200 A (LG HOUSHOLD & HEALTH CARE LTD (KR)) 22 October 2003 (22.10.2003) see claims 1-3, table 2, page 4 test result	1-2,4-6,8-10,13,16,19-20
X	JP 63179812 A (SHISEIDO CO LTD (JP)) 23 July 1988 (23.07.1988) see column 1 line 12, column 7 example 3	1-5,8,13,16-19

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

☒ See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 27 September 2010 (27.09.2010)	Date of mailing of the international search report <b>21 Oct. 2010 (21.10.2010)</b>
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/000983

### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16-22  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 16-22 are directed to a method of treatment of the human/animal body (PCT Rule 39.1(iv)), the search has been carried out and based on the medicament manufacturing use of said compositions.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.

PCT/CN2010/000983

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN 1923199 A	07.03.2007	None	
CN 1182788 A	27.05.1998	None	
KR 20030082200 A	22.10.2003	None	
JP 63179812 A	23.07.1988	None	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/000983

## A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4375 (2006.01)i

A61K 9/00 (2006.01)i

A61K 9/06 (2006.01)i

A61K 9/08 (2006.01)i

A61P 17/00 (2006.01)i

A61P 17/06 (2006.01)i

A61P 17/08 (2006.01)i

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