CLERA

Purpose for anti-irritant agent

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CLERA is derived from nature as an anti-irritant agent. It is developed for those who are craving for more clear & radiance skin and the relieving stressed skin of city life.

CLERA is for reducing skin irritation, notably that irritation attributed to the topical application of certain active species onto the skin, or due to exposure to the environment.

Forsythia suspensa fruit, Saururus chinensis Baill, Morus Alba Linne are well-known traditional Korean medicine. And we examined the effects of 1300 plant extracts that have been used Korean traditional herb medicine, and discovered 3 plants that Forsythia suspensa fruit, Saururus chinensis, Morus alba extract.

CLERA is a plant extract complex of Korean traditional medicine which has a good anti-inflammation effect. We have showed anti-inflammation effect from in vitro test and showed anti-irritation effect from in vivo test. It can application of various cosmetics formulations because we have widely tested its safety and stability.
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Part I. General Information
1. About the *Forsythia suspensa*

What is *Forsythia suspensa*?

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Korean forsythia fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin Name</td>
<td><em>Forsythia suspensa</em></td>
</tr>
<tr>
<td>INCI Name</td>
<td>Forsythia Suspensa Fruit Extract</td>
</tr>
<tr>
<td>Main ingredient</td>
<td>Saponin, Flavonoid, Alkaloid, Oleanolic Acid</td>
</tr>
<tr>
<td>Biological effects</td>
<td>Antioxidant activity, Antibacterial activity, anti-inflammatory</td>
</tr>
</tbody>
</table>

The fruit of *Forsythia suspensa* (Thunb.) is a well-known traditional Korean medicine, named “연교 [youn kyo]” in Korean. *Forsythia suspensa* (Thunb.) has been used widely in traditional medicines to treat gonorrhea, erysipelas, inflammation, pyrexia and ulcer. It has also shown antioxidant activity, as well as antibacterial, antiviral, choleric and antiemetic effects.

**Research data of *Forsythia suspense***

1. **Antioxidant and antibacterial activity**

   Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from *Forsythia suspensa*. J Pharm Pharmacol. 2008, 60(2):261-6

   **Abstract**: *Forsythia suspensa* (Thunb.) Vahl. has been widely used in traditional medicines in Asia to treat gonorrhea, erysipelas, inflammation, pyrexia, ulcer and other diseases. Recently the investigation has been focused on the antioxidant and antibacterial activity of this plant. However, limited scientifically proven information is available. We isolated two compounds (forsythiaside and forsythin) from this plant. The aims of this investigation, therefore, were to assay antioxidant activity and antibacterial properties of the two main and distinctive compounds isolated and to exploit antioxidants and antibacterial agents from natural compounds. The antioxidant activity was estimated using the 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity method and the in-vitro antimicrobial activity was evaluated by microtitre plate method. Forsythiaside was found to possess strong antioxidant and antibacterial activity but forsythin was much weaker. Owing to these properties, the study can be further extended to exploit the possible application of forsythiaside as an alternative antioxidant and antibacterial agent of natural origin.
2. Anti-inflammatory effect


Abstract; The enzymes 5-lipoxygenase and elastase are therapeutic targets in dermatological disorders such as psoriasis. Fifteen extracts from traditional Chinese medicinal plants used to treat topical inflammations were screened for their inhibitory effect on lipoxygenase, cyclooxygenase and elastase activity in intact leukocytes and platelets. Astragalus membranaceus, Forsythia suspensa and Poria cocos inhibited 5-lipoxygenase, with IC50 values of 141, 80 and 141 microg mL(-1), respectively. The latter two species, along with Angelica dahurica and Angelica pubescens, also inhibited elastase (IC50 values of 80, 123, 68 and 93 microg mL(-1), respectively), while A. pubescens, Atractylodes macrocephala, Lentinus edodes, Rehmannia glutinosa and Paeonia lactiflora selectively inhibited 12-(S)-HHTrE production, a valid marker of cyclooxygenase activity. The inhibition of phospholipase A(2) activity by P. cocos is discussed. Dehydrotumulosic and pachymic acids, which have been isolated from P. cocos, were shown to inhibit leukotriene B(4) release. The results indicate that both P. cocos and F. suspensa are potentially valuable species in the management of skin pathologies involving chronic inflammation.
2. About the *Saururus chinensis* Baill

**What is *Saururus chinensis* (Lour.) Baill?**

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chinese Lizardtail</th>
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</thead>
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<tr>
<td>Latin Name</td>
<td><em>Saururus chinensis</em> (Lour.) Baill.</td>
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<tr>
<td>INCI Name</td>
<td>Saururus Chinensis leaf Extract</td>
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<tr>
<td>Main ingredient</td>
<td>Quercetin, Methyl ethyl ketone, Lignoid manassantin, Saucernetin-8</td>
</tr>
<tr>
<td>Biological effects</td>
<td>anti-pyretic, anti-inflammatory, Atopic dermatitis-inhibitory effects, Anti-asthmatic activity</td>
</tr>
</tbody>
</table>

The *Saururus chinensis* is a well-known traditional Korean medicine, named "삼백초 [Sam Beak Cho]" in Korean. *Saururus chinensis* (Saururaceae) is a perennial herbaceous plant with potential therapeutic utility in treatment of various diseases such as edema, jaundice, gonorrhea, anti-pyretic, diuretic, and anti-inflammatory agents in Korean folk medicine.

**Research data of *Saururus chinensis* (Lour.) Baill**

1. **Atopic dermatitis-inhibitory effects**


*Abstract:* The present study was performed to examine whether the leaves of *Saururus chinensis* (LOUR.) BAILL (SC), an herb used for the management of various skin diseases including atopic dermatitis (AD) in Eastern countries, inhibited the development of AD-like skin lesions in NC/Nga mice which was induced by repeated application of picryl chloride (PiCl). The efficacy of SC was judged by measurement of skin severity, itching behavior, histological study, serum IgE levels, IL-4 and IFN-gamma in lymph nodes. Oral administration of SC extract to the PiCl-treated NC/Nga mice for 8 weeks (5 d per week) inhibited significantly the development of AD-like skin lesions macroscopically. Histologically, SC inhibited dermatitis changes like hypertrophy, hyperkeratosis, and infiltration of inflammatory cells into epidermis and dermis. The itching behavior and serum IgE level decreased significantly after SC administration. SC administration enhanced IFN-gamma mRNA expression but did not have an effect on IL-4 mRNA expression. These results suggest that SC could inhibit the
development of AD-like skin lesions in NC/Nga mice possibly through modulating the Th1/Th2 imbalance by the promoting of Th1 cell response. Thus, SC may be an alternative substance for the management of AD patients.

2. Anti-asthmatic activity


Abstract; As an attempt to find bioactive medicinal herbs exerting anti-asthmatic activity, the effects of an ethanol extract from the parts of Saururus chinensis were evaluated in both in vitro and in vivo. The ethanol extract of S. chinensis (ESC) inhibited generation of the cyclooxygenase-2 (COX-2) dependent phases of prostaglandin D(2) in bone marrow-derived mast cells in a concentration-dependent manner with an IC(50) value of 14.3 microg/ml. ESC also inhibited leukotriene C(4) production with an IC(50) value of 0.3 microg/ml. This demonstrates that ESC has COX-2/5-lipoxygenase dual inhibitory activity. In addition, this compound inhibited degranulation reaction in a dose dependent manner, with an IC(50) value of 1.3 microg/ml. An ovalbumin induced mouse asthmatic animal model was used to determine its in vivo anti-asthmatic activity. The oral administration (50-200 mg/kg) of ESC reduced the number of infiltrated eosinophil in a bronchoalveolar lavage fluid. Furthermore, ESC (100 mg/kg) inhibited the eotaxin and IL-4 mRNA expression levels. These results suggest that the anti-asthmatic activity of S. chinensis might in part occur via the inhibition of eicosanoid generation, degranulation as well as the down regulation of IL-4 and eotaxin mRNA expression.
3. About the *Morus Alba Linne*

**What is Morus Alba Linne?**

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<th>Common Name</th>
<th>Mulberry, Mori Cortex</th>
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<td>Latin Name</td>
<td><em>Morus Alba Linne</em></td>
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<tr>
<td>INCI Name</td>
<td>Morus Alba root Extract</td>
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<td>Main ingredients</td>
<td>Campesterol, Quercetin, Oxyresveratrol, Resveratrol, Betulinic acid, Mulberroside A</td>
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<tr>
<td>Biological effects</td>
<td>antiphlogistic, liver protective, kidney protective, antioxidative effect, antiviral effect, whitening effect</td>
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</table>

The *Morus alba* is a well-known traditional Korean medicine, named "상백피[Sang Beak Pi]" in Korean. Mulberry (*Morus alba* L., Moraceae) is cultivated in China, Japan and Korea, and their leaves have been used for a long time to feed silkworms (Bombyx mori L.). The root bark of mulberry tree (*Morus alba* L.) has been used in traditional Korean medicine (Oriental medicine) as antiphlogistic, liver protective, kidney protective, hypotensive, diuretic, anti-pyretic and analgesic agent.

**Research data of Morus Alba Linne**

1. Anti-oxidant effect

Quantitative analysis of aglycone quercetin in mulberry leaves (*Morus alba* L.) by capillary zone electrophoresis. Electrophoresis. 2003, 24(7-8):1236-41

*Abstract*: A capillary zone electrophoresis method was established for analysis of aglycone quercetin in mulberry leaves (*Morus alba* L.). The influence of, e.g., background electrolyte concentrations and pH, surfactant concentrations, organic solvents, temperature, and voltage on the separation of aglycone quercetin, rutin, quercitrin, kaempferol, catechin, and gallic acid was systematically investigated. The optimum condition providing baseline separation of all compounds within 16.5 min was obtained in 150 mM boric acid (pH 10.0) using a fused-silica capillary with an effective length of 42.5 cm (50 microm inner diameter), temperature of 32 degrees C, and voltage of 15 kV. Method assessment was performed by standard addition method using rutin as an internal standard. Linearity of the method was excellent ($r^2 > 0.999$) over the concentration tested (40-160 microg/mL). The relative standard deviations (%RSDs) from injection, intraday, and interday precision were less than 2.5%. Recoveries were good (asymptotically equal to 100.0%,%RSD = 0.8%) with a limit of detection (LOD) and limit of quantitation (LOQ) of 0.86 and 3.16
microg/mL (%RSD = 1.8%), respectively. The aglycone quercetin found in the mulberry leaves was 0.452 g/100 g (%RSD = 0.6%) on dry weight.

2. Whitening effect


*Abstract*: The current study was carried out to investigate the in vitro effects of an 85% methanol extract of dried *Morus alba* leaves on melanin biosynthesis, which is closely related to hyperpigmentation. These extracts inhibited the tyrosinase activity that converts dopa to dopachrome in the biosynthetic process of melanin. *Mulberroside F* (moracin M-6, 3’-di-O-beta-D-glucopyranoside), which was obtained after the bioactivity-guided fractionation of the extracts, showed inhibitory effects on tyrosinase activity and on the melanin formation of melan-a cells. This compound also exhibited superoxide scavenging activity that is involved in the protection against auto-oxidation. But its activity was low and was weaker than that of kojic acid. These results suggest that mulberroside F isolated from mulberry leaves might be used as a skin whitening agent.
4. Skin Disorders Caused By Cosmetics

1. IRRITANT CONTACT DERMATITIS

This is the commonest skin disorder that can arise from the use of cosmetics. The most frequent presenting complaint is facial itch and rash.

Types of cosmetics causing irritant contact dermatitis include:

A. **CLEANSERS**: Facial cleansers which often contain surfactants that are necessary to facilitate proper cleansing of the skin.

B. **TONERS AND ASTRINGENTS**: Toners and astringents may contain alcohol or acids, like alpha-hydroxyacid (AHA). Some patients may develop skin problems from these products.

C. **FACIAL TREATMENT**: Facial treatment involves the use of manual manipulation and application of chemicals on the skin, often resulting in exfoliation of the upper skin surface. Mild irritation is inevitable, but severe skin inflammation, like dermatitis can occur

What are the symptoms and sign irritation; Itchiness, Redness, Swelling and later scaling and peeling of the skin can occur.

2. ALLERGIC CONTACT DERMATITIS

Another important skin disorder caused by cosmetics is allergic contact dermatitis. It occurs less frequently than irritant contact dermatitis. It is often difficult to differentiate between an allergic from an irritant contact dermatitis by the appearance of the rash alone. Cosmetics ingredients that can cause skin allergy include *fragrances, preservatives and sunscreens*.

What are the symptoms and sign irritation; itchiness, redness, swelling or even blistering on the affected skin.
5. Mediator of Inflammation

Although inflammation has long been known as a localized protective reaction of tissue to irritation, injury, or infection, characterized by pain, redness, swelling, and sometimes loss of function, there has been a new realization about its role in a wide variety of diseases, including cancer. While acute inflammation is a part of the defense response, chronic inflammation can lead to cancer, diabetes, cardiovascular, pulmonary, and neurological diseases.

![Diagram of inflammation and its role in tumorigenesis](image)

Fig. Different faces of inflammation and its role in tumorigenesis.
1. **Cyclooxygenase (COX)-2**, an inducible enzyme with expression regulated by NF-κB. COX-2, the inducible isoform of prostaglandin H synthase, has been implicated in the growth and progression of a variety of human inflammations. Prostaglandin E2 (PGE₂) plays crucial roles in various biological events such as neuronal function, female reproduction, vascular hypertension, tumorigenesis, kidney function and inflammation.

2. **5-Lipoxygenase (5-LOX)** is a key enzyme in the metabolism of arachidonic acid to leukotrienes. Several studies suggest that there is a link between 5-LOX and carcinogenesis in humans and animals. In addition to the important role of leukotrienes as mediators in allergy and inflammation.

3. **Inducible nitric oxide-synthase (iNOS)** is one of three key enzymes generating nitric oxide (NO) from the amino acid l-arginine. iNOS gene expression and subsequent mRNA translation is controlled by various agonists, especially pro-inflammatory mediators. The most prominent cytokines involved in iNOS stimulation are TNF-α, IL-1β, and IFN-γ.

4. **TNF, interleukins, chemokines, COX-2, 5-LOX, and iNOS are all regulated by the transcription factor NF-κB (Nuclear factor-kB).** Although this factor is expressed in an inactive state in most cells, cancer cells express an activated form of NF-κB. This activation is induced by a wide variety of inflammatory stimuli and carcinogens, and the gene products regulated by it mediate tumorigenesis.
Part Ⅱ. Technical Data
1. Explain of CLERA

Overall Procedure for the preparation of CLERA

- Material purchase and also certificate of origin & quality
- Grinding whole dried plants
- Extraction and then 1st Filtration (remove the extracted plants)
- Evaporation (remove the EtOH)
- Centrifugation and then 2nd Filtration (remove the precipitate)
- Measurement of dry content on 110℃

- Re-extraction
  - > Dry contents 1%
    - Yes: Produce of final product and sterilization
    - No: Quality control
      - Yes: Product packaging ready for market
      - No: Re-extraction

Extraction Solvent: 70% EtOH (natural origin)
Extraction time: 12hr
Extraction temperature: 20–25℃
Residual solvent (EtOH): less than 1%
### CLERA

**Specification**

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Forsythia suspense / Saururus chinensis Baill / Morus Alba Linne</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCI Name</td>
<td>Water / Butylene glycol / Forsythia Suspensa Fruit Extract / Saururus Chinensis leaf Extract / Morus Alba Root Extract</td>
</tr>
<tr>
<td>Colour</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Odor</td>
<td>Typical</td>
</tr>
<tr>
<td>pH (10% solution)</td>
<td>4.0 - 7.0</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>0.980-1.100</td>
</tr>
<tr>
<td>Heavy Metal</td>
<td>≤ 20ppm</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>≤ 2ppm</td>
</tr>
<tr>
<td>Dry residue (110 °C)</td>
<td>0.1% (± 0.01)</td>
</tr>
<tr>
<td>Distillated water</td>
<td>less than 40%</td>
</tr>
<tr>
<td>1,3 - Butylene glycol contents</td>
<td>60%</td>
</tr>
<tr>
<td>Preservative</td>
<td>Non-added</td>
</tr>
<tr>
<td>Residual solvent (EtOH)</td>
<td>less than 1%</td>
</tr>
<tr>
<td>EINECS (Butylene glycol)</td>
<td>203-529-7</td>
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<tr>
<td>CAS (Butylene glycol)</td>
<td>107-88-0</td>
</tr>
</tbody>
</table>

**Microbiology**

- **Total Aerobic Count**: Less than 100 cfu/g
- **E. coli**: Not detected
- **Salmonella**: Not detected

**Storage condition**

Sealed containers should be stored at a temperature of 10-30°C (50-86°F). Quality might be affected after opening packing, please refer to MSDS for more informations. After opening the drums, sterilization is no more guaranteed.

**Packaging unit**

5kg / 10kg / 20kg

**Recommendation dosage**

0.5% ~ 2%

**Expiration Date**

2 years in sealed original packing, stored in due conditions

**Application**

Cosmetics; skin, lotion, cream, mask pack, essence, massage cream etc
Body care, Hair care, Baby goods, Personal care
2. Stability study

Stability as a function of pH

We have evaluated the stability of CLERA at different pH, temperature, ethanol, at its recommended dosage 1% to 3% in final products.

Function of pH of CLERA solution was measured using the potentiometric method. Measurements were made at room temperature within a pH range of 2 to 10.

<table>
<thead>
<tr>
<th>pH V/V</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>3%</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ ; stable, ±; slightly unstable

CLERA shows good stability within the tested range of pH. Accordingly, it allows the use in any formulations, we suggest to formulate in the respect of the skin’s pH.
Stability as a function of temperature

This study was made at the pH of the solution (pH close to 5.0) at temperatures ranging from 40 to 80°C, for 2 hours.

<table>
<thead>
<tr>
<th>Temp V/V</th>
<th>40°C</th>
<th>60°C</th>
<th>80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30min</td>
<td>60min</td>
<td>120min</td>
</tr>
<tr>
<td>1%</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3%</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5%</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+; stable, ±; slightly unstable

CLR shows good stability within the tested range of temperature. Therefore, it allows the use in any formulations, CLR can be included at any step of production process without any problem for stability.
Stability in the presence of ethanol

The study of solubility in various water/ethanol mixtures was made at room temperature at the pH of the solution (pH close to 5.0)

<table>
<thead>
<tr>
<th>(V/V)</th>
<th>Ethanol / H₂O (V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/90</td>
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<tr>
<td>1%</td>
<td>+</td>
</tr>
<tr>
<td>3%</td>
<td>+</td>
</tr>
<tr>
<td>5%</td>
<td>+</td>
</tr>
</tbody>
</table>

+ ; stable, ±; slightly unstable

CLERA shows good stability within 10/90 (v/v) ~ 60/40(v/v) the 1% tested concentration and also shows good stability within 10/90 (v/v) ~ 40/60(v/v) the 3% tested concentration. The 5% tested concentration shows slightly unstable above 30/70(v/v). Therefore, it allows the use in any formulations, excepted considering limitations of ethanol in final products.
3. Safety study

Repeated insult patch test (RIPT)

OBJECTIVE
To determine the irritation and/or sensitization potential of a test material after repeated application under occlusive, semi-occlusive or open patches to the skin of human subjects.

TEST MATERIAL
CLERA 10% solution in distilled water

STUDY DATES
This study was initiated on June 25th, 2007 and was completed on July 20th, 2007

PANEL SELECTION
Panels of human subjects, male and female, randomly selected. No individuals were empanelled if they exhibited or had a history of acute or chronic dermatologic, medical, or physical conditions that could interfere with dermal scoring.

TEST METHOD
Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction period. The subjects remove the patches 24 hours after each application. 24 hour rest periods follow each removal. Prior to each reapplication, site(s) were graded for dermal irritation and sensitization

Dermal scores
0 No visible skin reaction
± Barely perceptible erythema (minimal)
1+ Mild erythema (diffuse)
2+ Well defined erythema
3+ Erythema and oedema
4+ Erythema and oedema with vesiculation

Ten to 21 days after application of the final induction patch, challenge patch(es) are applied to previously unpatched sites, adjacent to the original induction patch sites. The challenge sites 24~72 hours after application.

REFERENCE
Standard Operating Procedures, Clinical Trials 930.00, Repeat Insult Patch Test (RIPT)
# RESULT

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<td>0</td>
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<td>10</td>
<td>LHS</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

# CONCLUSION

Based on the test population of 10 subjects and under the conditions of this study, the CLERA 10% solution identified did not demonstrate a potential for eliciting dermal irritation on sensitization.
Ames test for mutagenicity

OBJECTIVE
To screen for mutagens through the simple and inexpensive procedure that uses a bacterial test organism. It is a biological assay used in genetics, generally genetic toxicology, to test for mutagenic properties of a chemical compound.

STUDY DATES
This study was initiated on July 25th, 2007 and was completed on July 29th, 2007

TEST ORGANISMS
The test organism is a histidine-negative (his⁻) auxotrophic strain of *salmonella typhimurium* that will not grow on a medium deficient in histidine unless a back mutation to his⁺ (histidine-positive) has occurred.

PRINCIPLE OF TEST METHOD
It is recognized that the mutagenic effect of a product is frequently influenced by the enzymatic pathway of an organism, whereby non-mutagens are transformed into mutagens and vice versa when introduced into human system.

REFERENCE

RESULT

<table>
<thead>
<tr>
<th>Test samples</th>
<th>His⁺ revertants/plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>Spontaneous test</td>
<td>20</td>
</tr>
<tr>
<td>4-NQO</td>
<td>3000</td>
</tr>
<tr>
<td><strong>CLERA</strong> 30%</td>
<td>54</td>
</tr>
<tr>
<td><strong>CLERA</strong> 50%</td>
<td>43</td>
</tr>
<tr>
<td><strong>CLERA</strong> 70%</td>
<td>31</td>
</tr>
</tbody>
</table>

CONCLUSION
Based on the test procedure and under the conditions of this study, the **CLERA** from 30% to 70% solution identified did not demonstrate a potential for mutagenicity.
MATERIAL SAFETY DATA SHEET

DATE: April 30, 2007
WRITTEN BY:
SIGNATURE:

1. PRODUCT AND COMPANY IDENTIFICATION

Product name: CLERA
Product code: RA020
Use: Raw material for cosmetic
Manufacturer: RADIANT Rm 207 BIOINDUSTRY FOUNDATION, 198-53
Hupyeong-Dong, Chunchon-city, Gangwon-Do, ROK
Tel: +82-33-244-1243
Fax: +82-33-244-1367
E-mail: cmo@eadiant.co.kr
Emergency call +82-33-244-1243

G2. COMPOSITION AND INFORMATION OF INGREDIENTS

Chemical Name: None
Molecular weight: N.A
CAS Number (1,3 Butylene glycol): 107-88-0
EINECS (1,3 Butylene glycol): 203-529-7
INCI Name: Water (and) Butylene glycol (and) Forsythia Suspensa Fruit Extract (and) Saururus Chinensis leaf Extract (and) Morus Alba Root Extract
Hazardous ingredients: None

3. HAZARDS

Information provided on the health effects of this product is based on individual components. All ingredients are bound and potential for hazardous exposure as shipped is minimal. However, some vapours may be released upon heating and the end-user (fabricator) must take the necessary precautions (mechanical ventilation, respiratory protection, etc) to protect employees from exposure.

Main hazards: No Known Health hazards
Health risks: Experience shows no acute irritancy or toxic effects.
Environment risks: Handle the product with good working practice avoiding dispersion into the environment.
Routes of exposure: Inhalation, Ingestion, Skin/Eye contact.

4. FIRST AID

Skin: Wash off with soap and plenty of water.
Eyes: Immediately irrigate with water.
Ingestion: Do not induce vomiting without medical advice.
Inhalation: Move to fresh air in case of accidental inhalation of fumes from overheating or combustion. When symptoms persist or in all cases of doubt seek medical advice.

5. FIRE FIGHTING

Suitable extinguishing media: Water spray, dry powder.
### Unsuitable extinguishing media
- Carbon monoxide (CO)
- Carbon dioxide (CO₂)

### Special fire-fighting procedures
- None known

### Unusual fire/explosion hazards
- Full-face self-contained breathing apparatus (SCBA) used in positive pressure mode should be worn to prevent inhalation of vapour/fumes.
- May emit irritant/toxic vapour/fumes under fire conditions.

### 6. ACCIDENTAL RELEASE

Remove heat and sources of ignition. Drums and packing in danger should be cooled by pulverized water, as heating could provoke a rise in pressure with explosion or deflagration risks.

Prevent entry into watercourses and pipeworks.

Immediately mop up with suitable absorbent equipment for subsequent correct disposal according to current legislation.

### 7. HANDLING AND STORAGE

Store in a dry place and away from light to insure the quality. Keep the drums well closed in a well aired place.

Keep away from heat and sources of ignition. Do not smoke. In case of important heating of the liquid, there is a risk of formation of explosive mixtures with air. Risk of fire in case of contact with hot area, sparks or flames.

### 8. EXPOSURE CONTROL AND PERSONAL PROTECTION

<table>
<thead>
<tr>
<th>Respiratory protection</th>
<th>Facial mask</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand Protection :</td>
<td>Protective gloves</td>
</tr>
<tr>
<td>Eye Protection :</td>
<td>Glasses with air-tight protection</td>
</tr>
<tr>
<td>Skin and body protection :</td>
<td>Long sleeved clothing and safety shoes</td>
</tr>
<tr>
<td>Engineering measures</td>
<td>Heat only in areas with appropriate exhaust ventilation. Provide appropriate exhaust ventilation at machinery.</td>
</tr>
</tbody>
</table>

### 9. PHYSICAL AND CHEMICAL PROPERTIES

| Appearance: | Liquid |
| Colours:    | Light Yellow Color |
| Odour:      | Typical |
| Specific Gravity at 20°C: | 0.980-1.100 |
| Solubility in water: | Soluble |
| pH(soln.):  | 4.00 – 7.00 |
| Vapour pressure | N.A. |
| Vapour density | N.A. |

### 10. STABILITY AND REACTIVITY

**Thermal decomposition:** Distillation without decomposition at normal pressure. No thermal decomposition in case of correct storage and handling.

**Dangerous decomposition products:** No dangerous decomposition products if storage and handling conditions are respected. In case of fire or thermal decomposition, release of carbon monoxide and carbonic anhydride.

**Dangerous reactions:** Reacts violently with powerful oxidising agents.

**Hazardous decomposition products:** May emit irritant/toxic vapour/fumes under fire conditions.
11. TOXICOLOGY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute toxicity</td>
<td>No toxical effect known</td>
</tr>
<tr>
<td>Sensitisation</td>
<td>Not sensitizing</td>
</tr>
<tr>
<td>Inhalation</td>
<td>Inhalation is possible only under aerosol conditions, and vapour/fumes might be irritant/toxic.</td>
</tr>
</tbody>
</table>

12. ECOLOGICAL INFORMATION

No ecologic effect known

13. DISPOSAL

Recommended method to dispose the product without danger: Dispose in accordance with the current legislation preferably using high temperature incineration or In a biological purification station in accordance with the current legislation.

14. TRANSPORT INFORMATION

Not dangerous for transport. (ROAD-RAIL, SEA, AIR)

15. REGULATORY INFORMATION

Labelling according E.E.C. directives : Not submitted to labelling

16. OTHER INFORMATION

This information is furnished without warranty, except that it is accurate to the best knowledge of RADIANT INC. The data on this sheet relates only to the specific material designated herein.

### Remarks column

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMO statement</td>
<td>To the best of our knowledge CLERA is not made from nor contains any ingredients derived from GMO sources.</td>
</tr>
<tr>
<td>Animal testing declaration</td>
<td>CLERA has not at any time been tested on animals other than consenting human subjects.</td>
</tr>
</tbody>
</table>
Part III. Efficacy Data
1. Anti-inflammation effect (In-vitro test)

**OBJECTIVE**

CLERA is composed of Korean traditional medicines that have long been used as an anti-inflammation treatment. In order to investigate the possibility of CLERA as a cosmetic ingredient, we measured its anti-inflammation effect by inhibition of iNOS protein expression and inhibition of NO produce.

**REFERENCE**

Inhibition of methanol extract from the aerial parts of Saururus chinensis on lipopolysaccharide-induced nitric oxide and prostaglandin E$_2$ production from murine macrophage RAW 264.7 cells. Biol Pharm Bull. 2003, 26(4):481-6.

**STUDY DATES**

This study was initiated on April 12$^{th}$, 2007 and was completed on April 19$^{th}$, 2007

**POSITIVE CONTROL**

Portulaca oleracea L. extract, N$^6$-monomethyl-L-arginine (NMMA)

**TESTING MATERIALS**

CLERA (0.01%~5%)

**TESTING METHOD**

**Cell culture and Sample treatment** - The RAW 264.7 murine macrophage cell line was obtained from the Korea Cell Line Bank. These cells were grown at 37$^{\circ}$C in DMEM medium supplemented with 10% FBS, penicillin and streptomycin sulfate in a humidified atmosphere of 5% CO2. Cells were incubated with CLERA at increasing concentrations (0.01-5%) and simulated with LPS 100ng/ml for 24h.

**Nitrite assay (NO assay)** - Nitrite accumulation, an indicator of NO synthesis, was measured in the culture medium by the Griess reaction. Briefly, 100$\mu$l of cell culture medium was mixed with 100$\mu$l of Griess reagent, incubated at room temperature for 10min, and then absorbance at 550nm was measured in a ELISA.

**Western blot assay** - Macrophages were collected and the protein content was determined. Equal amounts of protein(15mg/lane) were loaded and electrophoresed on a 10% SDS-polyacrylamide gel. After the fractionated protein was blotted onto a nitrocellulose membrane, the membrane was incubated over night in blocking buffer (5% nonfat dry milk, 10 mM Tris(pH 7.5), 100 mM NaCl, 0.1% Tween 20) and then treated with a mouse monoclonal COX-2 antibody for 1h. After washing, the membrane was incubated with a horseradish peroxidase-conjugated anti-mouse IgG antibody. To detect iNOS the membrane was treated with a monoclonal iNOS antibody.

For CLEAn & RAdiance
RESULT

Effect of *Forsythia suspensa* Fruit, *Saururus chinensis*, *Morus alba* extract on nitrite production by LPS-induced RAW 264.7 Cells.

<table>
<thead>
<tr>
<th>Test materials</th>
<th>Inhibition Concentration 50 (IC₅₀) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMMA (NO synthesis inhibitory agent)</td>
<td>6.96</td>
</tr>
<tr>
<td><em>Saururus chinensis</em> extract</td>
<td>10.31</td>
</tr>
<tr>
<td><em>Morus alba</em> extract</td>
<td>9.55</td>
</tr>
<tr>
<td><em>Forsythia suspensa</em> fruit extract</td>
<td>8.89</td>
</tr>
</tbody>
</table>

We examined the effects of 1300 plant extracts that have been used Korean traditional medicine, and discovered 3 plants that *Forsythia suspensa* Fruit, *Saururus chinensis*, *Morus alba* extract had the most potent anti-irritant effect. We have tested about the inhibition of NO produced effect of each test materials compare with NMMA as a positive control. As a result, all of the 3 plants had a good NO inhibition like as NMMA.

Effect of CLERA on NO production by LPS-induced RAW 264.7 Cells.

For CLEan & RAdiance
CONCLUSION

To determine the effects of CLERA on NO production in RAW 264.7 cells, the cell were treated with LPS (100ng/ml) induced approximately 9-fold greater NO production compared with control (data not shown), and this induction was inhibited by CLERA in a dose-dependent manner. And also to determine if the inhibitory effect of CLERA on these inflammatory mediators was examined their expression levels by western blot. In response to LPS, the expression level of iNOS was markedly augmented, and CLERA significantly inhibited the iNOS protein induction in a dose-dependent manner.
2. Anti-Skin Irritation effect (In vivo test)

**OBJECTIVE**
The aim of the study was to evaluate short-term effects of use CLERA on SLS-irritated human skin. Sodium lauryl sulfate (SLS), a surfactant frequently used in the induction of experimental irritant contact dermatitis in animals and in humans, characteristically induces a dose-related increase in erythema index (EI) and TEWL (transepidermal water loss). The investigation was comparing with positive control both using double-blind randomized study design.

*In vivo induction of erythema and TEWL after application of 1% SLS - 1% SDS were applied on normal human skin during 24 hr patch test. Erythema and TEWL scores were obtained 24 hr after removal of the patch.*

**REFERENCE**

**STUDY DATES**
This study was initiated on May 10th, 2007 and was completed on May 15th, 2007

**TESTING METHOD**
Methods SLS (1% v/v) was applied under occlusion on the inner arm of 10 healthy volunteers for 24 h. Subsequently, the test areas were treated with CLERA(1% v/v) and Portulaca oleracea L. extract(1% v/v) as a positive control and distilled water as a negative control. After treated these samples, we checked erythema index by Mexameter in a time dependent manner served as readout parameters to assess the SLS-induced skin irritation.
RESULT

We have tested irritant patch test for development of anti-irritant materials. We have treated SLS as an irritant, 24 hours later we treatment with CLERA and *Portulaca oleracea* L. extract. For detected the erythema index (EI) we used Mexameter (MX 18, Courage-Khazaka, Germany). As seen in the data presented CLERA is capable of providing protective affects against stress from environment and CLERA has better efficacy if compared with *Portulaca oleracea* L. extract as a positive control. In SLS-induced skin irritation model in vivo, we found to reduce skin erythema and improve barrier recovery. We can explain from this data CLERA helps to inhibit the production of NO (Nitric Oxide) and iNOS reducing the inflammatory response in the skin and thereby potentially reducing subclinical irritation.

<table>
<thead>
<tr>
<th></th>
<th>0-8hr</th>
<th>8-24hr</th>
<th>0-24hr (Δ delta value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td><em>Portulaca oleracea</em> L. extract</td>
<td>41.3</td>
<td>20</td>
<td>61.3</td>
</tr>
<tr>
<td>CLERA</td>
<td>48.4</td>
<td>18.0</td>
<td>66.5</td>
</tr>
</tbody>
</table>

CONCLUSION

We have tested irritant patch test for development of anti-irritant materials. We have treated SLS as an irritant, 24 hours later we treatment with CLERA and *Portulaca oleracea* L. extract. For detected the erythema index (EI) we used Mexameter (MX 18, Courage-Khazaka, Germany). As seen in the data presented CLERA is capable of providing protective affects against stress from environment and CLERA has better efficacy if compared with *Portulaca oleracea* L. extract as a positive control. In SLS-induced skin irritation model in vivo, we found to reduce skin erythema and improve barrier recovery. We can explain from this data CLERA helps to inhibit the production of NO (Nitric Oxide) and iNOS reducing the inflammatory response in the skin and thereby potentially reducing subclinical irritation.
For *Forsythia suspensa* Fruit. (*J Pharm Pharmacol. 2003, 55(9):1275-82*)

5-Lipoxygenase and HLE inhibition effect of *Forsythia suspensa* Fruit.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>5-Lipoxygenase</th>
<th></th>
<th></th>
<th>HLE release</th>
<th>HLE activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LTB₄</td>
<td>5-(S)-HETE</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 6</td>
<td>100 ± 9</td>
<td>100 ± 14</td>
<td>80 ± 12</td>
<td>104 ± 1</td>
</tr>
<tr>
<td><em>Forsythia suspensa</em> (200 µg/ml)</td>
<td>12 ± 19</td>
<td>60 ± 16</td>
<td>24 ± 10</td>
<td>9 ± 4</td>
<td>28 ± 1</td>
</tr>
</tbody>
</table>

Effect of *Forsythia suspensa* (200 µg/ml) on the production of 5-lipoxygenase metabolites and HLE (human leukocyte elastase) release and activity. As a result F.suspendea was the most potent inhibitor of both 5-lipoxygenase and HLE activity. The production of LTB₄ and its all-trans-isomers was decreased, while the inhibition of 5-(S)-HETE remained insignificant. *If the blocking of both 5-lipoxygenase and HLE enzymes is considered a primary objective in dermatological disorders.*
Effect of *Saururus chinensis* Baill on PGE$_2$ production and COX-2 protein expression by LPS-induced RAW 264.7 cells. (SCB; *Saururus chinensis* Baill)

To examine whether the SCB could inhibit PGE$_2$ production, cells were pre-incubated with the SCM for 1 h, and then activated with 1m g/ml LPS. The production of PGE$_2$ was significantly inhibited by the SCB in a dosedependent manner. PGE$_2$ production due to attenuation of iNOS and COX-2 protein expression.

Inhibition of LPS-Induced NF-k B Activation by the SCB(*Saururus chinensis* Baill)

The expression of iNOS and COX-2 in murine macrophages has been shown to be dependent on NF-k B activation. The possibility that SCB may inhibit the activity of NF-k B was examined. The results indicate that SCB inhibition of expression of both iNOS and COX-2 proteins and mRNA was most likely due to SCB suppression of NF-kB.
Part IV. Conclusion
1. Summary

Purposed intracellular signaling pathways for the anti-irritant effect action of CLERA

**IRRITANT**
(LPS, SLS, Retinol, surfactant, preservative etc)

- Inos
- COX-1
- COX-2
- LOX-5

- NO
- PGE$_2$
- LTB$_4$

**Skin irritation (inflammation)**
(swelling, redness, warmth, pain, etc)

*For CLEan & RAdiance*
2. Application of cosmetics
Certifications

- May. 2005 Received an ISO 9001/14001
- August. 2005 Certified clean manufacturing company
- Member of International Trade Association
- Member of Cosmetic, Toiletry and Fragrance Association
- Selected as the promising small or medium sized business enterprise designated by Gangwon Province

Rm 207 Bioindustry Innovation center
Hi-Tech Venture Town, 198-53
Hupyeng, Chuncheon, Gangwon
Seoul, Korea 200-957
Email: radiantcmo@chol.com
www.eradiant.co.kr