HI-CLEA

Purpose for anti-irritant agent

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

In our daily lives, human skin and scalp are damaged by various kinds of harmful factors inducing irritation of skin or scalp. Sometimes skin care product and hair care product induce the irritation or allergy to our skin, in some cases it would be severe problems.

**HI-CLERA** is derived from one of the Korean traditional medicinal herb, Cynanchi Radix.

**HI-CLERA** takes care of key factors related to inflammation and also recovers the damaged skin irritated by stimulators in cosmetics. We have examined anti-inflammation effect from in vitro test and also examined anti-irritation effect from in vivo test.

**HI-CLERA** can be applied to various kinds of cosmetics with its great activity and stability and safety.

[Patent: KP 10-1127156]

Cosmetic composition containing 2,4-dihydroxyacetophenone or the extract of Cynanchi atratum Bunge.

Comprising 2,4-dihydroxyacetophenone with the usage of skin-irritation alleviation.
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Part I. General Information
1. **About Cynanchi Radix**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Cynanchi Radix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin name</td>
<td>Cynanchum atratum Bunge</td>
</tr>
<tr>
<td>INCI name</td>
<td>Cynanchum Paniculatum Extract</td>
</tr>
<tr>
<td>Korean name</td>
<td>백미(白薇, Bak Mi)</td>
</tr>
<tr>
<td>Main Ingredient</td>
<td>2,4-dihydroxyacetophenone, cynatratosides, glaucosides, atratosides</td>
</tr>
<tr>
<td>Biological Effect</td>
<td>Anti-inflammation, anti-irritation</td>
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</tbody>
</table>

**性平**（一云寒）味苦酸無毒治百邪鬼魅忽忽睡不知人狂惑邪氣寒熱溫瘧
生原野莖葉俱靑頗類柳葉根黃白色類牛膝而短小三月三日採根陰乾米泔浸去鬚蒸用 [本草]

*It has cold-property; its taste is salty and has no poison.*

*It is good for forgetfulness, hypnolepsy and also good for fever and cold.*

*It is grown in the field and wilderness, its stem and leaves are green as like willow leaves, its root is yellowish white and small and short. [Boncho]*
2. Research data of

Anti-inflammation effect

Water extract of Cynanchi atrati Radix regulates inflammation and apoptotic cell death through suppression of IKK-mediated NF-κB signaling (Department of Pharmacology, Research Institute for Medical Science, Daejeon Regional Cancer Center, College of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea, 2011)

Abstract;
ETHNOPHARMACOLOGICAL RELEVANCE: Cynanchi atrati Radix has been traditionally used as an anti-inflammatory agent to treat febrile diseases, acute urinary infection or subcutaneous pyogenic infection with invasion of the pathogenic factors.

AIM OF STUDY: Nuclear factor (NF)-κB is a pleiotropic transcriptional factor of many genes involved in inflammatory and anti-apoptotic responses. To identify a novel, potent inhibitor of NF-κB signaling pathway, a plant extract library of traditional oriental medicine was screened for the capability to block the NF-κB activity in cells overexpressing toll-like receptor 4 (TLR4), and then evaluated the anti-inflammatory and pro-apoptotic functions of water extract of Cynanchi atrati Radix (WECR) in macrophages and cancer cells, respectively.

MATERIALS AND METHODS: The effect of WECR on the proinflammatory mediators (inducible NO synthase [iNOS], cyclooxygenase [COX]-2), IkB-α degradation, RelA/p65 phosphorylation and caspase cleavages were measured by immunoblotting. NF-κB transcriptional activity, IkB kinase (IKK) activity and nitric oxide (NO) production was measured using the luciferase assay, in vitro kinase assay and Griess reaction.

RESULTS: WECR efficiently inhibited LPS-induced expression of proinflammatory mediators including iNOS and COX-2. IKK kinase activity, IkB-α degradation, nuclear translocation of RelA/p65 and NF-κB transcriptional activity induced by LPS were suppressed by WECR. Furthermore, WECR dramatically enhances the apoptotic response, as evident by the combination with tumor necrosis factor (TNF) was able to induce the cytotoxic action through caspase-dependent pathway.

CONCLUSION: These results indicate that WECR has a potential to inhibit IKK-mediated NF-κB activation, and is a valuable compound for modulating inflammatory or cancerous conditions.
Anti-febrile and anti-inflammatory effect

Antifebrile and anti-inflammatory effects of radix Cynanchi atrati (Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine, Beijing, 1995)

Abstract: The water extract of Radix Cynanchi Atrati used as intraperitoneal injection has been proved to have an obvious antifebrile++ effect on rat fever caused by 15% yeast suspension hypodermic injection as well as a significant anti-inflammatory effect. But the antiferbrile effect of its ethanol extract is not clear.

Anti-inflammatory and anti-nociceptive effect

The anti-inflammatory and anti-nociceptive effects of ethyl acetate fraction of cynanchi paniculati radix. (Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Iksan, Jeonbuk, Korea, 2006)

Abstract: The anti-inflammatory and anti-nociceptive effects and sedative activities of the ethyl acetate fraction of Cynanchum paniculatum (EACP) were evaluated in mice and rats by acetic acid-induced vascular permeability, arachidonic acid-induced paw edema, cotton pellet-induced granuloma formation, formalin-induced licking time, acetic acid-induced writhing response, and pentobarbital-induced sleeping time. EACP at a dose of 40 mg/kg significantly exhibited anti-inflammatory activities on acetic acid-induced vascular permeability, arachidonic acid-induced paw edema, and the late phase of formalin-induced licking time. Moreover, it showed anti-nociceptive effects on acetic acid-induced writhing responses and significant sedative effects on pentobarbital-induced sleeping time. The results demonstrated that the anti-nociceptive effects are apparently related to the sedative effects of EACP. These results support the use of Cynanchum paniculatum in relieving inflammatory pain.
3. 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.

An irritant directly damages cells if in contact with the skin in sufficient concentration and for sufficient time. Most irritants cause dermatitis by gradually overwhelming the skin's barrier and repair mechanisms. Mild irritants such as detergents will wash out the stratum corneum lipids and if exposure exceeds the capacity of the skin to regenerate those lipids, dermatitis will result. Powerful irritants - such as caustic soda - produce an immediate effect. These cause direct damage to keratinocytes which results in tissue death. Dermatitis induced by mild irritants is called chronic or cumulative irritant contact dermatitis.
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.

Both irritant and allergic contact dermatitis can occur together (particularly on the hands) and either may co-exist with constitutional dermatitis. It is common for exposure to occur to more than one irritant and more than one allergen at any one time. Such exposures may give rise to a cumulative irritant and cumulative allergic response. An irritant contact dermatitis may also develop first, rendering the skin more susceptible to penetration by sensitisers. It is also possible that an original allergic contact dermatitis might be later sustained by an irritant.
3. Adverse Effect of Hair Dye

People have been dyeing their hair for centuries. The great advantage of changing one's hair, as opposed to other parts of the body, is that it is an easy and non-invasive way of enhancing your appearance. But, hair coloring involves the use of chemicals capable of removing, replacing and/or covering up pigments naturally found inside the hair shaft.

Use of these chemicals can result in a range of adverse effects, including temporary skin irritation and allergy, hair breakage, skin discoloration and unexpected hair color results. Additionally, there is ongoing debate regarding more serious health consequences of hair color usage, including lead poisoning.

The use of hair dye can result in allergic reaction and skin irritation. Symptoms of these reactions can include redness, sores, itching, burning sensation and discomfort.
4. **Mediators of Skin Irritation**

1. **Prostaglandin E₂ (PGE₂)**
   Prostaglandin E₂ (PGE₂) is a primary product of arachidonic metabolism and is synthesized via the cyclooxygenase (COX) and prostaglandin synthase pathways. PGE₂ production is a commonly used method for the detection of COX-1 and COX-2 modulation and prostaglandin synthases.

2. **Tumor necrosis factor-α (TNF-α)**
   Tumor necrosis factor (TNF) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by other cell types as well. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, to induce apoptotic cell death, to induce sepsis (through IL1 & IL6 production), to induce cachexia, induce inflammation, and to inhibit tumorigenesis and viral replication.

3. **Interleukin-1 (IL-1)**
   Interleukin 1 is responsible for the production of inflammation, as well as the promotion of fever and sepsis. IL-1α inhibitors are being developed to interrupt those processes and treat diseases. IL-1α is produced mainly by activated macrophages, as well as neutrophils, epithelial cells, and endothelial cells. It possesses metabolic, physiological, haematopoietic activities, and plays one of the central roles in the regulation of the immune responses. It binds to the interleukin-1 receptor. It is on the pathway that activates TNF-α.

4. **β-hexosaminidase**
   Mast cells play significant roles in allergic diseases when the skin is stressed. Upon activation by the high-affinity IgE receptors (FcRI), they release factors such as histamine, cytokines, chemokines that ultimately cause allergic responses. To demonstrate the effect of anti-stress on the skin, the release of β-hexosaminidase, histamine, and cytokine production is examined.
Part II. Technical Data
1. Explain of HI-CLERA

Overall Procedure for the preparation of HI-CLERA

Material purchase and also certificate of origin & quality

Grinding whole dried Cynanchi Radix

*Extraction and then 1st Filtration

Centrifugation (4500rpm, 30min and then 2nd Filtration (remove the precipitate)

Measurement of dry content on 110°C

Produce of final product and sterilization

Quality control

Yes

Yes

Product packaging ready for market

No

*Extraction with 70% EtOH
- Extraction time : 12hr
- Extraction temperature : 20~25°C
- Filtration: paper filtration (pore size: 1㎛) for remove precipitation and suspended particles
# HI-CLERA

## Specification

<table>
<thead>
<tr>
<th>INCI Name</th>
<th>Water / Butylene glycol / Cynanchum Paniculatum Extract</th>
</tr>
</thead>
<tbody>
<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>Identification Test</td>
<td>Red-purple ring</td>
</tr>
<tr>
<td>Heavy Metal</td>
<td>≤ 20ppm</td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>≤ 2ppm</td>
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<tr>
<td>Preservative</td>
<td>0.5% Phenoxyethanol</td>
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<tr>
<td>Residual solvent (EtOH)</td>
<td>less than 1%</td>
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<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>
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## Microbiology

<table>
<thead>
<tr>
<th></th>
<th>Less than 100 cfu/g</th>
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<tbody>
<tr>
<td>Total Aerobic Count</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Not detected</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

## Storage condition

Sealed containers should be stored at a temperature of 10~30 ℃ (50~86 ℉). 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

## Expiration Date

2 years in sealed original packing, stored in due conditions

## Recommendation dosage

0.1~2%

## 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 HI-CLERA at different pH, temperature, ethanol, at its recommended dosage 1% to 5% in final products.

Function of pH of HI-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>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<th>10</th>
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<tr>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+; stable, ±; slightly unstable

HI-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>
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<tbody>
<tr>
<td></td>
<td>30min</td>
<td>60min</td>
<td>120min</td>
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<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

*HI-CLERA shows good stability within the tested range of temperature. Therefore, it allows the use in any formulations, HI-CLERA 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>
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<tr>
<th>(V/V)</th>
<th>Ethanol / H₂O (V/V)</th>
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<tbody>
<tr>
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<td>10/90</td>
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<td>1%</td>
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<tr>
<td>3%</td>
<td>+</td>
</tr>
<tr>
<td>5%</td>
<td>+</td>
</tr>
</tbody>
</table>

+ ; stable, ±; slightly unstable

*HI-CLERA* shows good stability within the tested range of ethanol content. It means you don't care about limitation of ethanol. Therefore, it allows the use in any formulations in final products.
3. Stability 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
HI-CLERA 10% solution in distilled water

STUDY DATES
This study was initiated on December 8th, 2011 and was completed on January 6th, 2012.

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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<tr>
<th>Subject Number</th>
<th>Subject Initials</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>24hr</th>
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<tbody>
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</tbody>
</table>

**CONCLUSION**
Based on the test population of 10 subjects and under the conditions of this study, the HI-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 December 9th, 2011 and was completed on January 14th, 2012.

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>
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<tbody>
<tr>
<td></td>
<td>TA98</td>
</tr>
<tr>
<td>Spontaneous test</td>
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</tr>
<tr>
<td>4-NQO</td>
<td>2950</td>
</tr>
<tr>
<td>HI-CLERA 10%</td>
<td>43</td>
</tr>
</tbody>
</table>

CONCLUSION
Based on the test procedure and under the conditions of this study, the HI-CLERA 10% solution identified did not demonstrate a potential for mutagenicity.
## 1. PRODUCT AND COMPANY IDENTIFICATION

<table>
<thead>
<tr>
<th>Product name</th>
<th>HI-CLERA</th>
</tr>
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<tbody>
<tr>
<td>Product code</td>
<td>RA120</td>
</tr>
<tr>
<td>Use</td>
<td>Raw material for cosmetic</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>RADIANT 1143 G-tech Village Geodu-ri Dongnae-myeon Chuncheon Gangwon Republic of Korea</td>
</tr>
<tr>
<td>Tel</td>
<td>+82-33-244-1243</td>
</tr>
<tr>
<td>Fax</td>
<td>+82-33-244-1367</td>
</tr>
<tr>
<td>E-mail</td>
<td><a href="mailto:radiantcmo@chol.com">radiantcmo@chol.com</a></td>
</tr>
<tr>
<td>Emergency call</td>
<td>+82-33-244-1243</td>
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## G2. COMPOSITION AND INFORMATION OF INGREDIENTS

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<thead>
<tr>
<th>Chemical Name</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>N.A</td>
</tr>
<tr>
<td>CAS Number (Butylene Glycol)</td>
<td>107-88-0</td>
</tr>
<tr>
<td>EINECS (Butylene Glycol)</td>
<td>203-529-7</td>
</tr>
<tr>
<td>INCI Name</td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td>Butylene Glycol</td>
</tr>
<tr>
<td></td>
<td>Cynanchum Paniculatum Extract</td>
</tr>
</tbody>
</table>

| 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

- **NFPA:** Health: 1 Flammability: 1 Reactivity: Unsuitable extinguishing media
  - Flammable properties
    - Flash point (test method): N.A
  - Flammable limits in air, % by volume:
    - Upper: No Information Lower: No Information
  - Autoignition temperature: 394 °C (741 F)
  - Products of combustion: Carbon monoxide and butadiene.
  - Extinguishing Media:
    - Use alcohol type aqueous film forming foam for large fires. Use CO2 or dry chemical for small fires.
  - Fire Fighting Environmental Concerns:
    - Thoroughly decontaminate bunker gear and other fire-fighting equipment before re-use.
  - Fire Fighting Instructions:
    - Water spray should be used to cool fire-exposed structures and vessels. Water or foam may cause frothing. Water spray can be used to reduce the intensity of flames and to dilute spills to a non-flammable mixture. Keep personnel removed from and upwind of fire. If potential for exposure to vapors or products of combustion exists, wear full fire fighting turnout gear and NIOSH approved self-contained breathing apparatus. Oxidizing chemicals may accelerate the burning rate in a fire situation.

### 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 absorbant 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

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

### 9. PHYSICAL AND CHEMICAL PROPERTIES
### Appearance:
- Liquid

### Colours:
- Light Yellow

### Odour:
- Typical

### Specific Gravity at 20 °C:
- 0.980-1.100

### Solubility in water:
- Soluble

### pH(10% soln.):
- 4.00 – 7.00

### Vapour pressure:
- N.A.

### Vapour density:
- N.A

### Preservative:
- 0.5% Phenoxyethanol.

### 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

**Acute toxicity:** No toxic effect known

**Sensitisation:** Not sensitizing

**Inhalation:** Inhalation is possible only under aerosol conditions, and vapour/fumes might be irritant/toxic.

### 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>GMO statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>To the best of our knowledge HI-CLERA is not made from nor contains any ingredients derived from GMO sources.</td>
</tr>
</tbody>
</table>
Part III. Efficacy Data
1. Anti-inflammatory Activity

OBJECTIVE

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

REFERENCE


STUDY DATES

This study was initiated on September 12th, 2011 and was completed on September 26th, 2011

POSITIVE CONTROL

⁰-G-monomethyl-L-arginine(NMMA) : Inhibitor of nitric oxide synthase (It is used as a biochemical tool in the study of physiological role of nitric oxide)

TESTING MATERIALS

Cynanchum Paniculatum Extract (0.1 ~ 5 %, x 10⁻⁴)

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 °C in DMEM medium supplemented with 10% FBS, penicillin and streptomycin sulfate in a humidified atmosphere of 5% CO₂. Cells were incubated with Cynanchi Radix extract at increasing concentrations (0.5~5%, x 10⁻³) and stimulated 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 µl of cell culture medium was mixed with 100 µl 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 (15 mg/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 overnight 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 1 h. 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.

**Cytokine expression assay** – Macrophages were collected and the cytokine content was determined using PGE2, kit (R&D system, KEG004), Mouse TNF-α (Ebioscience, Cat. 88-7324-22), Mouse IL-1α/IL-1 F1 (R&D system DY400)

**RESULT**

**a. Effect of Cynanchum Paniculatum Extract on nitrite production by LPS-induced RAW 264.7 Cells.**

<table>
<thead>
<tr>
<th>Test materials</th>
<th>Concentration (% × 10^-4)</th>
<th>Inhibitory activity of NO production</th>
<th>IC50 (% × 10^-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynanchum Paniculatum Extract</td>
<td>0.1</td>
<td>8.3 ± 1.2</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>11.8 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15.5 ± 2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38.5 ± 3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>77.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>NMMA</td>
<td>0.5</td>
<td></td>
<td>6.96</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10.8 ± 10.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>39.8 ± 7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>58.3 ± 5.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>73.5 ± 4.8</td>
<td></td>
</tr>
</tbody>
</table>

We have tested about the inhibitory activity of NO production of Cynanchum Paniculatum Extract compare to NMMA as a positive control. As a result, HI-CLERA had a superior activity than NMMA.
b. *Effect of Cynanchum Paniculatum Extract on LPS-induced iNOS protein expression*

HI-CLERA inhibits the expression of iNOS protein in a dose dependent manner at 0.5–5% (x 10⁻⁴).

![Image showing Cynanchi Radix Extract (% x 10⁻⁴) and LPS inhibition of iNOS protein expression](image)

Cynanchum Paniculatum Extract (% x 10⁻⁴) | Cytokine Inhibitory Activity (%) | PGE₂ | TNF-α | IL-1 |
--- | --- | --- | --- | --- |
0.5 | - | - | 15.1 ± 0.2 |
1 | - | - | 20.2 ± 1.8 |
3 | 32.0 ± 2.2 | 5.1 ± 3.3 | 28.9 ± 4.0 |
5 | 47.1 ± 1.9 | 10.9 ± 4.1 | 86.1 ± 6.3 |

Cynanchum Paniculatum Extract inhibits the production of PGE₂ and TNF-α which are the inflammatory cytokines. IL-1 (Interleukin 1) is a regulatory factor of inflammation. When LPS was treated to the cells as a stimulator, IL-1 is produced in the cells. With the treatment of Cynanchum Paniculatum Extract, IL-1 contents were getting lower in a dose dependent manner. This result indicates the anti-inflammatory effect of Cynanchum Paniculatum Extract.
**d. Comparison between HI-CLERA and CLERA on NO production by LPS-induced RAW 264.7 Cells.**

![Graph showing inhibitory activity of NO production by CLERA and HI-CLERA](image)

* CLERA: Forsythia Suspensa Fruit Extract, Saururus Chinensis leaf extract, Morus Alba Root Extract

We additionally have tested the anti-inflammatory activity of HI-CLERA compared to CLERA. It is found that HI-CLERA inhibits the NO production rate (%) compared to CLERA about 2 times higher. Thus, HI-CLERA can be a superior anti-inflammatory ingredient.

**CONCLUSION**

To determine the effects of HI-CLERA on NO production in RAW 264.7 cells, the cells were treated with LPS. Compared to the positive control (NMMA), Cynanchi Radix Extract showed the superior activity on inhibition of NO production. And also to determine if the inhibitory effect of HI-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 Cynanchi Radix extract significantly inhibited the iNOS protein induction in a dose–dependent manner. To determine the cytokine inhibitory activity, PGE2, TNF-α, IL-1 were used, and Cynanchi Radix extract inhibits all these cytokine effectively. And in the comparison of activity between CLERA and HI-CLERA, HI-CLERA showed the superior activity than CLERA.
2. *Inhibition activity against Scalp Irritation*

**OBJECTIVE**

MEA (Monoethanolamine) is a stimulator of hair dye. In order to investigate the possibility of HI-CLERA as a cosmetic ingredient, we measured its inhibitory activity against scalp irritation induced by MEA.

**STUDY DATES**

This study was initiated on September 12th, 2011 and was completed on September 26th, 2011.

**NEGATIVE CONTROL**

- 0.1% MEA (Monoethanolamine)

**TESTING MATERIALS**

Cynanchum Paniculatum Extract (0.5 ~ 2%, x 10⁻³)

**TESTING METHOD**

*MTT assay* – We used human keratinocyte (HaCaT) and MTT (Thiazoly blue Tetra-zolium Bromide, Sigma, M2128) for test. HaCaT was incubated in 37°C, CO₂ incubator. Cynanchi Radix extract was treated to HaCaT for 24hr and afterwards MTT was treated for 4hr. Cell viability was measured compared with the amount of formazan produced by untreated control cells.
When 0.1% of MEA was treated to the cells, cell viability was about 50%. With the treatment of Cynanchum Paniculatum Extract, cell viability was getting higher. This indicates the Cell protective effect of Cynanchi Radix Extract.

CONCLUSION
To determine the effects of HI-CLERA on inhibitory activity against scalp irritation, MEA was treated to the cell. With the treatment of Cynanchum Paniculatum Extract, cell viability was increased in a dose dependent manner. Thus it is found that Cynanchum Paniculatum Extract inhibits the Scalp irritation induced by Hair dye.
3. Anti-stress Activity

OBJECTIVE
The aim of the study was to evaluate anti-stress activity of HI-CLERA on β-hexosaminidase assay.

STUDY DATES
This study was initiated on September 12th, 2011 and was completed on September 26th, 2011

TESTING MATERIALS
Cynanchum Paniculatum Extract (0.1~1%, x 10^-3), Quercetin(5%, x 10^-3)

TESTING METHOD
RBL-2H3 cell was incubated in 37°C, CO2 incubator. for 24hr. and samples were pre-treated to the cell for 1hr. 200ul/ml of DNP-BSA were added to Tyrode’s buffer and incubated for 1hr. Transfer the medium to 96-well plate and add substrate and incubate fir 1hr. After adding the lysis buffer, medium was transferred to another 96-well plate and add substrate and then incubate for 1hr. Add stop buffer and measure the absorbance at 405nm.

RESULT

![Graph showing the inhibition of β-hexosaminidase release rate(%) in a dose dependent manner]

CONCLUSION
As a result of the test, Cynanchum Paniculatum Extract had shown the inhibition of β-hexosaminidase release rate(%) in a dose dependent manner. Through the β-hexosaminidase release rate, we can estimate the histamine release rate indirectly. This result indicates that Cynanchum Paniculatum Extract have a anti-stress activity including the anti-allergic activity.
4. **Anti-skin irritation against Retinol (In vivo)**

**OBJECTIVE**
The aim of the study was to evaluate short-term effects of use HI-CLERA on Retinol-irritated human skin.

**STUDY DATES**
This study was initiated on September 16th, 2011 and was completed on September 17th, 2011

**TESTING MATERIALS**
Retinol(1%), HI-CLERA (1~2%) 

**TESTING METHOD**
Retinol (1% v/v) was applied under occlusion on the inner arm of 20 healthy volunteers for 24 h. Subsequently, the test areas were treated with HI-CLERA(1~2%). After treated these samples, we checked erythema index by Mexameter in a time dependent manner served as readout parameters to assess the Retinol-induced skin irritation.

**RESULT**

<table>
<thead>
<tr>
<th>Erythema index (EI)</th>
<th>HI-CLERA</th>
<th>Placebo (No treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>164.23 ± 24.17</td>
</tr>
<tr>
<td>Retinol treated (1%)</td>
<td></td>
<td>387.45 ± 30.47</td>
</tr>
<tr>
<td>After 24hr</td>
<td>313.90 ± 23.08</td>
<td>301.38 ± 14.80</td>
</tr>
<tr>
<td>After 48hr</td>
<td>258.22 ± 17.92</td>
<td>247.20 ± 43.92</td>
</tr>
<tr>
<td>Skin recovery effect (%)</td>
<td>58.6</td>
<td>63.1</td>
</tr>
</tbody>
</table>

**CONCLUSION**
Despite many beneficial effects on dermatological applications, retinol cause severe local irritation. After treated with HI-CLERA as a 1% and 2% its skin recovery effect was very good than placebo treated group.
5. Anti-skin irritation induced by shampoo (In vivo)

OBJECTIVE
Various kinds of hair care product like shampoo, hair dye, conditioner contains harmful factors (MEA, PPD, etc) irritating the scalp and human skin. To evaluate the anti-skin irritation induced by hair care product, shampoo with HI-CLERA was treated to the skin, and was evaluated its recovery activity.

STUDY DATES
This study was initiated on October 10th, 2011 and was completed on October 17th, 2011

TESTING MATERIALS
Shampoo (containing HI-CLERA(0.5~2%) and surfactant)

TESTING METHOD
Shampoo with HI-CLERA(0.5~2%) was applied under occlusion on the inner arm of 4 healthy volunteers for 24 h. After treated these samples, we checked erythma index for 0 day, 4 day.

RESULT

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HI-CLERA 0.5%</th>
<th>HI-CLERA 1.0%</th>
<th>HI-CLERA 2.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Erythma Index Graph]

- 0 day
- 4 day

Control
HI-CLERA 0.5%
HI-CLERA 1.0%
HI-CLERA 2.0%
CONCLUSION
Main stimulating factor of shampoo is surfactant like as SLS, ALS, AOS, etc, and it induces the skin erythma and epidermal water loss of the skin. After treatment of shampoo containing HI-CLERA, erythma was shown to all group. During 0~4 days after treatment, HI-CLERA-treated group has shown the anti-irritation activity in a dose dependent manner compared to control group.

[Comparison between HI-CLERA and CLERA on anti-irritation activity].

SLS was treated to the skin and the skin recovery activity of CLERA and HI-CLERA was evaluated. After treatment, Skin recovery effect of HI-CLERA was higher than CLERA in 0~8hr, and the total skin recovery effect during 0~24hr, HI-CLERA showed the excellent activity than CLERA. This indicate that HI-CLERA has superior activity of anti-irritation induced by SLS than CLERA effectively in short period.

* Skin Recovery Effect(%) 

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>CLERA (1%)</th>
<th>HI-CLERA (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-8hr</td>
<td>5</td>
<td>48.4</td>
<td>65.6</td>
</tr>
<tr>
<td>8-24hr</td>
<td>28</td>
<td>18</td>
<td>9.4</td>
</tr>
<tr>
<td>0-24hr (Δ delta value)</td>
<td>33</td>
<td>66.5</td>
<td>75</td>
</tr>
</tbody>
</table>
6. HI-CLERA compare with α-bisabolol, DPG

OBJECTIVE

To determine the anti-irritation effect of HI-CLERA, compare with commercial anti-irritation ingredient, α-bisabolol, DPG (Dipotassium Glycyrrhizinate)

TEST MATERIAL

HI-CLERA 1, 2% solution in distilled water / RA12101-120518
α-bisabolol 0.2, 0.5% solution in distilled water / Sigma, cat.# 14462
DPG (Dipotassium Glycyrrhizinate) 0.2, 2% solution in distilled water / TCI cat.# G0270

STUDY DATES

This study was initiated on July 5th, 2012 and was completed on July 10th, 2012

TEST METHOD

SDS (3% v/v) was applied under occlusion on the inner arm of 4 healthy volunteers for 24 h. Subsequently, the test areas were treated with HI-CLERA, α-bisabolol, DPG (Dipotassium Glycyrrhizinate). After treated these samples, we checked erythema index by Mexameter and Transepidermal water loss by Tewameter in a time dependent manner served as readout parameters to assess the SDS-induced skin irritation.

RESULT

1. Skin recovery effect – Erythma

<table>
<thead>
<tr>
<th></th>
<th>Irritation</th>
<th>Treated of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>DPG 0.2%</td>
<td>100</td>
<td>76.3</td>
</tr>
<tr>
<td>DPG 2%</td>
<td>100</td>
<td>53.2</td>
</tr>
<tr>
<td>α-bisabolol 0.2%</td>
<td>100</td>
<td>96.7</td>
</tr>
<tr>
<td>α-bisabolol 0.5%</td>
<td>100</td>
<td>94.2</td>
</tr>
<tr>
<td>HI-CLERA 1%</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>HI-CLERA 2%</td>
<td>100</td>
<td>69.8</td>
</tr>
</tbody>
</table>

DPG 2% > HI-CLERA 2% > HI-CLERA 1% > DPG 0.2% > α-bisabolol 0.5% > α-bisabolol 0.2%
2. Skin recovery effect – TEWL

![Graph showing skin recovery effect - TEWL](image)

DPG 2% > DPG 0.2% > \( \alpha \)-bisabolol 0.5% > Hi-CLERA 2% > bisabolol 0.2% > Hi-CLERA 1%

**CONCLUSION**

- All 3 kind of tested material have good anti-skin irritation effect against SLS irritation.
- DPG : 0.2~2% have a good effect for both Erythma and TEWL
- \( \alpha \)-bisabolol : 0.2~0.5% have a good effect for TEWL, otherwise it showed low effect for Erythma
- Hi-CLERA : 1~2% have good effect for both TEWL and Erythma like as DPG
- From this study we discovered that substitute Hi-CLERA for DPG, \( \alpha \)-bisabolol in various kinds of cosmetics.
Part IV. Conclusion
To keep our skin / scalp clean and healthy, we need to get rid of harmful factors like Nitric oxide as an inflammation mediator. Our skin / scalp are damaged by some skin care products or hair care products in daily lives and get weaker and weaker. By the stimulators, our skin / scalp has suffered like contact dermatitis, allergic reactions, etc.

One of the Korean traditional herb, *Cynanchum Paniculatum Extract* has great activity of anti-inflammation and anti-irritation against inflammatory mediators or irritation stimulators. It effectively inhibits the NO production, iNOS expression, IL-1, TNF-α, PGE2 expression, β-hexosaminidase. And it also recovers the damaged skin irritated by MEA, SLS, etc.

Compared to CLERA, *HI-CLERA* has superior activity regarding its anti-inflammation / anti-irritation activities, thus it can be a **extra Anti-irritation agent.**
2. Application of Cosmetics

HI-CLERA can be applied to various kinds of cosmetics like skin care, hair care, body care, etc.
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 Gang won Province
- Selected as the Promising Export Firm by small & medium business administration

Thank You

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Radiant
Free radicals & Antioxidants