ORYZA OIL & FAT CHEMICAL CO., LTD
ver. 1.1 HS
1. Introduction

Rheumatism and knee osteoarthritis (usually referred to as “arthropathy”) are common degenerative disorders of aging. The early stage of rheumatism is characterized by the inflammation of genetic and immunologic origins of the synovial membrane which lines the joint capsules. As inflammation progresses, synovial fluid pools, pannus (increased synovial membrane) is formed followed by destruction of cartilage. At the advance stage, joints are filled with synovial villus tissue without proper functioning. Upon progression of symptoms, patients will experience severe pain, joints deformities coupled with mental stress. The conventional treatment prescribed for rheumatism includes: Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), steroids, SH compounds and immunosuppressive agents. The commonly prescribed latest NSAIDS group - Cyclooxygenase-2 (COX-2) selective agents usually require 1-3 months to be effective while posting side effects on hepatic functions & hypoactive immune system. On the other hand, osteoarthritis is characterized by the deterioration of cartilage in the joints (i.e., intersections of two bones), resulting in pain and loss of function. The condition primarily affects weight-bearing joints such as the knees, hips, feet, and back, and the joints in the fingers and hands. As the disease progresses, crevices and bone spurs (osteophytes) may develop within the affected joint, increasing pain and decreasing mobility. NSAIDS are commonly prescribed for pain relief while nutritional supplement (e.g. chondroitin, glucosamine) may be used as part of an overall treatment program to reduce symptoms of osteoarthritis.

Fig. 1. Pathophysiology of Arthritis
It is estimated that there are more than 750,000 arthropathic people in Japan and 6.6 million in the US. Nutritional supplements (e.g. glucosamine) are commonly used for the prevention and pain relief. However, there is insufficient research data to prove the benefits of this treatment. In addition, animal derived Type II collagen has been used in the treatment of rheumatism as Cartilage types II collagen was strongly expressed in late-stage specimens, reflecting the high matrix-remodelling activity of advanced osteoarthritic cartilage. Other commonly used supplements include chondroitin sulphate, hyaluronic acid, methylsulfonyl methane (MSM), cat’s claw (TNF-α & PGE₂ supressor) and white willow.

Ginger (*Zingiber Officinale* Roscoe) is commonly found in OTC preparations as an aromatic stomachic, anti-nausea and pain relief remedies. Actives found in ginger essential oil include gingerols and shogaols ¹ which are vanilloids ² that bind to capsaicin-sensitive parasympathetic nerve ³. The pharmacological properties of these compounds has been identified to be anti-inflammatory that suppress platelet aggregation ⁴ and nitric oxide production ⁵ while inhibit COX-2 activity ⁶.

Red Ginger (*Z. officinale* var. Rubra) is a variance of the *Zingiber Officinale* species cultivated in Indonesia and Malaysia. Besides having gingerols and shogaols, it is loaded with anthocyanin and tannin in its root bark. Red Ginger is reddish-violet in colour and known as “Jahe Merah” by the natives (Fig. 2). Traditionally, it is used in spices, syrup and as remedy for rheumatism, osteoporosis, asthma & cough. Recent reports on the anti-inflammatory effect ⁷-⁹ of anthocyanin of *Z. Officinale* received much attention. Oryza Oil & Fat Chemical Co., Ltd. prompted researches into evaluating the anti-inflammatory effect of Red Ginger with various experimental models and results revealed that aqueous ethanol extract of Red Ginger is beneficial against acute and chronic inflammations. In addition, human study revealed that Red Ginger Extract lowered serum hyaluronic acid, hence prevents destruction of joint cellular matrix component.

Red Ginger Extract is an all natural analgesic & anti-inflammatory agent suitable for the application of anti-arthritis preparations.
References


2. Physiological Compounds of RED GINGER EXTRACT

Common to the *Z. Officinale* family, Red Ginger Extract is rich in gingerols & shogaols as illustrated in Fig. 3. Meanwhile, [6]-gingerol and 3R,5S-[6]-gingerdial are characteristic compounds of Red ginger Extract due to its high concentration as compared to other *Z. Officinale* species.

![Diagram showing various physiological compounds of Red Ginger Extract](image)

Fig. 3. Physiological Compounds of Red Ginger Extract
3. Anti-arthritis effect RED GINGER EXTRACT

(1) Effect on acute and chronic inflammation

1) Acetic Acid Induced Abdominal Inflammation in Mice

The effect of Red Ginger Extract (non binder, [6]-gingerol 2%, tannin 2%; this preparation is used in the following experiments) on acute inflammation was experimented on acetic acid induced abdominal inflammation model in mice. The analgesic effect of Red Ginger Extract was determined by Writhing Counts.

As illustrated in Fig. 4, oral administration of Red Ginger Extract (10 – 100mg/kg) demonstrated a dose dependent pain relief effect with significant reduction in Writhing counts. Similarly, it exerted a dose-dependent anti-inflammatory effect where the amount of leaked dye, an indicator on extend of inflammation, reduced significantly at 50 and 100mg/kg. In addition, Young H-Y et al., showed that intraperitoneal injection of [6]-gingerol (50mg/kg) relieved pain by 50% †). As revealed in this experiment, Red Ginger Extract in low oral dose of 0.2 – 2mg/kg demonstrated effective analgesic and anti-inflammatory effects suggesting the synergisms of various physiological compounds presents in Red Ginger Extract.

Fig. 4. The effect of Red Ginger Extract on acetic acid induced abdominal inflammation in mice.
(Upper graph: The Analgesic Effect of Red Ginger Extract on Writhing counts
Lower graph: The Anti-inflammatory Effect of Red Ginger Extract)
Each column represents the mean value with S.E. (n=12).
“*” denotes significant difference from control where *: p<0.05, **: p<0.01
[Method]
Red Ginger Extract was given orally to fasting mice (ddy, male, 5-wk old) followed by I.V. injection of Pontamin Sky Blue 2% (10mL/kg), an indicator of inflammation 55min later. Inflammation was induced 5min later by acetic acid 1% (10mL/kg) via intraperitoneal injection. Frequency of Writhing events was counted for 15min followed by dissection of abdominal cavity under ether anesthesia. Abdominal cavity containing dye of Pontamin Sky Blue was flushed with saline (8mL). Leaked dye was measured and calibrated to 10mL for absorbance measurement at wavelength 590nm.

2) Rat Adjuvant Arthritic Model
The effect of Red Ginger Extract on chronic inflammation was experimented using rat adjuvant arthritis model. This model is generally used for the diagnosis of rheumatism. As shown in Fig. 5, edema was induced and observed in rats treated with adjuvant (control). No significant changes observed in group treated with Red Ginger Extract 1mg/kg. However, marked anti-inflammatory effect was observed on day-13 in group treated with Red Ginger Extract 10mg/kg. Indomethacin (0.5mg/kg), a commonly prescribed NSAIDS drug was used as positive control, showing a significant anti-inflammatory effect (p<0.01) upon induction of inflammation throughout end of experiment.

![Fig. 5. The Effect of Red Ginger Extract (RGE) on Rat Adjuvant Arthritis Model.](image)

Each point represents the mean value with S.E. of 7 rats. “*” denotes differences from control, *: p<0.05; **: p<0.01 respectively.
X-ray images of limbs of rat treated with adjuvant were taken. Fig. 6 showed that severe joint destruction observed in control group with adjuvant while joint destruction in group treated with Red Ginger Extract & Indomethacin was less severe.

![X-ray images of limbs of rat treated with adjuvant.](image)

**Fig. 6. X-ray images of limbs of rat treated with adjuvant.**
Solid line: Edema Dashed line: destruction of joint

Joint specimens were examined under microscope and revealed evidence of joint destructions accrues where synovium thickens, the cartilage and the underlying bone begins to disintegrate (as in the control group). Meanwhile, less destruction observed in bone tissues of samples treated with Red Ginger Extract 10mg/kg (Fig. 7). Red Ginger Extract prevents autoimmune inflammatory disorders.

![Microscopic illustration of joint tissues of rats treated with adjuvant.](image)

**Fig. 7. Microscopic illustration of joint tissues of rats treated with adjuvant (H.E. stain, x10).**
Left: Control ① papillary growth of villus, ② Medium-level of bone destruction, ③ increased of osteoclast cells
Right: Sample treated with Red Ginger Extract (10mg/kg) ④ bone tissue was normal, less destruction
[Method]
S.C injection of 0.1ml Freund’s complete adjuvant (Difco, 1ml) containing inactivated strains of *Mycobacterium butyricum* (Difco, 10mg) was introduced to the rear of right limbs of rats (SD, male, 8 week old). Samples of Red Ginger Extract were given daily after immunization and edema was determined by measuring volume of limbs treated with adjuvant.

3) Arthritis Model of Joint Cellular Matrix in Mice
Further research was prompted to evaluate the effect of Red Ginger Extract on cellular matrix of arthritic joint in mice. Type II collagen was introduced as antigen in this model. Fig. 8 illustrated that inflammation reduced significantly (p<0.05) with increasing concentration of Red Ginger Extract.

![Graph showing the effect of Red Ginger Extract on cellular matrix of arthritic joints in mice.](image)

**Fig. 8. The Effect of Red Ginger Extract on Cellular Matrix of Arthritic Joints in Mice**
Each point represents the mean value with S.E. of 7 mice. “*” denotes significance difference from control, *: p<0.05

[Method]
Equal volume of Bovine Type II collagen and Freund’s Complete Adjuvant (100μL) was intradermally injected to the lower limbs of mice (male DBA/1J, 5-wk old). Above booster immunization was prepared similarly and re-introduced 3 weeks later. Mice were divided into 4 groups according to the levels of inflammation. Samples of Red Ginger Extract were given orally at different concentration (5, 10 & 20mg/kg) respectively on a daily basis. Meanwhile, 5% acacia gum suspension was used as control. Intensity of inflammation was determined every 3-4 days during 31-day of samples administration. Intensity of inflammation was determined according to Banerjee S *et al.* † as arthritis score in five levels based on average of total scores of 4 legs (16 at maximum).

(2) Anti-inflammatory effect of Red Ginger Extract – Mechanism of Actions

Inflammation is triggered in response to mechanical, chemical or immunological challenges. Cascade of inflammatory cells are released upon infiltration of macrophages into injured tissue (e.g. pro-inflammatory cytokines, prostaglandins [PG] ) resulting in pain, tenderness and swelling in the affected injured tissue. Further in-depth research was prompted to evaluate the effect of Red Ginger Extract on migration of macrophages, PG production and cyclo-oxygenase activity.

1) Inhibition of Macrophage Migration

The effect of Red Ginger Extract on the release of monocytes (which will differentiate into macrophage) was experimented with TAXIScan (Effector Cell Institute). As illustrated in Fig. 9 & 10, Red Ginger Extract demonstrated a dose-dependent suppression on the migration of monocytes of human peripheral blood which was stimulated by monocyte chemotactic factor (MCP-1, 10nm).

![Fig. 9. Migration of monocytes in chambers of TAXIScan](amorphous particles are cells)

[Method]

Crude fraction of monocytes was obtained from treatment of dextran & Lymphorep on human peripheral blood. Crude fraction of monocytes was suspended in a medium where CD13 & CD19 micro beads were added for placement in LD column (Myltenyi Biotec) for collection of the non-absorbing fraction – fraction of monocyte. Density of monocytes was adjusted to 2x10⁶ pieces/ml followed by the addition of Red Ginger Extract and incubation for 1 hour at 37°C. Migration of monocytes was triggered with MCP-1 (10nm) and results was observed in TAXIScan (Effector Cell Institute).
Fig. 10, The Effect of Red Ginger Extract on the migration of human monocytes triggered with MCP-1.
2) **Inhibition of prostaglandins E2(PGE2) production**

Further experiment was prompted to study the effect of Red Ginger Extract on PG production. Cultured macrophage cells (RAW264.7) were stimulated by lipopolysaccharide (LPS) to produce prostaglandins (PG). Results showed that Red Ginger Extract significantly suppressed the production of PGE2 from cells RAW264.7 at concentration of 3 & 10µg/ml. Meanwhile, no cytotoxicity occurred at these concentrations.

![Graph showing the effect of Red Ginger Extract on PGE2 production](image)

**Fig. 11. The effect of Red Ginger Extract on PGE2 production**

Each column represents the mean value with S.E. of 3 experiments. **“*** denotes significant difference from control. ***: p<0.01**

**[Method]**

RAW264.7 cells was suspended in MEM medium containing 0.1mM non-essential amino acids, 10% fetal bovine serum, penicillin (100units/mL) and streptomycin (100µg/mL) at concentration of 1x10^6 cells/mL. 200µl of the suspension was cultured in a 48-welled plate for 24 hours followed by rinsing in serum-free medium (200µl). Later, serum-free medium (170µl) was added to each well followed by the addition of 10µl LPS (200µg/ml; *E.coli* serotype 0127:B8, Sigma) solution and continue culture for 20 hours. Supernatant layer was collected upon completion of culture for the measurement of PGE2 with Prostaglandin E2 EIA Kit Monoclonal (Cayman Chemical Co.)
3) Selective Inhibition of COX-2
In-depth research was fostered to understand the anti-inflammatory effect of Red Ginger Extract by studying its effect on COX-1 & COX-2 *in-vitro*. As illustrated below, Red Ginger Extract exerted a dose-dependent (1 to 10µg/ml) effect on PG production with COX-1. In contrary, Red Ginger Extract suppressed PG production *in-vitro* at concentration of 3 and 10µg/ml.

The discovery on the stimulatory effect of Red Ginger Extract on COX-1 is indeed interesting. Kim *et al.* 1) revealed that [6]-gingerol enhances COX-2 expression in mouse skin stimulated by phorbol ester while exert no effect on COX-1. Alternatively, research leaded by Nurtjahja-Tjendraputra 2) revealed that [6]-gingerol or its related compounds inhibited platelet aggregation via its suppression on COX-1 activity. This indicates that besides gingerol, other physiological components of Red Ginger Extract enhanced the effect of COX-1 leading to the production of PG which is protective to the gastric membrane. Hence, it is believed that Red Ginger Extract exerts selective anti-inflammatory effect on COX-2 inhibition similar to that of NSAIDS while protective on gastric membrane.

![Fig. 12. The effect of Red Ginger Extract on COX-1 & COX-2.](image)
Each column represents the mean value of 2 experiments.


[Method]
Commerically available COX Inhibitor Screening Assay Kit (Cayman Chemical Co.) was used for the above experiments.

4) The effect of Red Ginger Extract on the inhibition of NO production
NO (nitric oxide) is produced by macrophage or damage cartilage cells upon injury. Experiments are prompted to study the effect of Red Ginger Extract on NO production from RAW264.7 cells triggered with LPS. Results showed that 100µg/ml of Red Ginger Extract suppressed the production of nitrogen monoxide by approximately 50%. It was found that [6]-shogaol and [6]-gingerdilols exerted potent inhibitory effect on NO production instead of its principal component, gingerols. Besides, tannin fraction (tannin 5.8%) isolated from Red Ginger Extract demonstrated similar inhibitory effect against NO production. Hence, the essential oil components and tannin of Red Ginger Extract are inhibitory against NO production by macrophage..

Table 1. The effect of Red Ginger Extract on the inhibition of NO production

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>0 (LPS-)</th>
<th>4.11 ± 0.40</th>
<th>3.87 ± 0.34</th>
<th>3.84 ± 0.23</th>
<th>4.98 ± 0.22</th>
<th>5.10 ± 0.24</th>
<th>4.98 ± 0.22</th>
<th>5.03 ± 0.45</th>
<th>4.98 ± 0.26</th>
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</thead>
<tbody>
<tr>
<td>Red Ginger Extract</td>
<td>1.00 ± 0.20</td>
<td>(7.8)</td>
<td>(8.8)</td>
<td>(9.8)</td>
<td>(16.9)</td>
<td>(19.5)</td>
<td>(16.9)</td>
<td>(19.5)</td>
<td>(16.9)</td>
</tr>
</tbody>
</table>


| Method | Commercially available COX Inhibitor Screening Assay Kit (Cayman Chemical Co.) was used for the above experiments. |
| Table 1. The effect of Red Ginger Extract on the inhibition of NO production | | | | | | | | | |
| Conc. (µg/mL) | 0 (LPS-) | 4.11 ± 0.40 | 3.87 ± 0.34 | 3.84 ± 0.23 | 4.98 ± 0.22 | 5.10 ± 0.24 | 4.98 ± 0.22 | 5.03 ± 0.45 | 4.98 ± 0.26 |
| Red Ginger Extract | 1.00 ± 0.20 | (7.8) | (8.8) | (9.8) | (16.9) | (19.5) | (16.9) | (19.5) | (16.9) |

| 4) The effect of Red Ginger Extract on the inhibition of NO production | NO (nitric oxide) is produced by macrophage or damage cartilage cells upon injury. Experiments are prompted to study the effect of Red Ginger Extract on NO production from RAW264.7 cells triggered with LPS. Results showed that 100µg/ml of Red Ginger Extract suppressed the production of nitrogen monoxide by approximately 50%. It was found that [6]-shogaol and [6]-gingerdilols exerted potent inhibitory effect on NO production instead of its principal component, gingerols. Besides, tannin fraction (tannin 5.8%) isolated from Red Ginger Extract demonstrated similar inhibitory effect against NO production. Hence, the essential oil components and tannin of Red Ginger Extract are inhibitory against NO production by macrophage. | | | | | | | | |
[Method]
200µl of pre-cultured RAW264.7 cells was incubated in 48-welled plate for 24-hour followed by change of medium to serum free medium. LPS (final concentration 20µM) and sample solution of Red Ginger Extract was added and continue cultured for another 24-hour. Last, 100µl of supernatant layer was collected for determination of NO contents using Grease reagent.

Based on the several findings above, Red Ginger Extract is anti-arthritis and anti-inflammatory via the following mechanisms:
1. Inhibition on migration of human monocytes into inflammatory tissues
2. Selective inhibition of COX-2 leading to the suppression of PG production
3. Inhibition of NO production

Besides, it is also suggestive that Red Ginger Extract regulates the functions of osteoclast cells which posses similar properties to that of macrophages.

Fig. 13.
The anti-arthritis effect of Red Ginger Extract – mechanism of actions & site of actions
4. The Effect of Red Ginger Extract on Inflammatory Parameters – Human Trial

Previous positive findings prompted an in-depth study on the effect of Red Ginger Extract on human. The effect of Red Ginger Extract on blood profile of arthritic patients was evaluated. Protocol of the study as follow:

No. of subjects: 9 male volunteers
Dosage: Red Ginger Extract 50mg/day
Duration: 28 days
Observation: Blood Profile Screening on blood protein, albumin, ratio A/G, hyaluronic acid, IgG, IgM, MMP-3 (matrix metalloprotein), blood platelet, ESR & CRP

As tabulated in Table 2, hyaluronic acid, blood platelet and C-reactive protein (CRP) levels were lowered with treatment of Red Ginger Extract. Reduction in hyaluronic acid was especially significant (p<0.05) under the treatment of Red Ginger Extract as its level return to the healthy normal range. Hyaluronic acid is produced by synovial cells of the joint cavity and usually detected at high concentrations in synovial fluid of rheumatic patients. Upon destruction of joint cavity, hyaluronic acid penetrates into the blood stream resulting in elevated blood hyaluronic acid level. Red Ginger Extract is beneficial in rheumatoid arthritis with its hyaluronic acid lowering effect.

Table 2. The effect of Red Ginger Extract on Blood Parameters of Arthritic Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before ingestion</th>
<th>After ingestion</th>
<th>Standard value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/dL)</td>
<td>7.1±0.3</td>
<td>7.2±0.4</td>
<td>6.5〜8.3</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>4.5±0.2</td>
<td>4.6±0.3</td>
<td>3.8〜5.7</td>
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<tr>
<td>A/G</td>
<td>1.7±0.2</td>
<td>1.7±0.2</td>
<td>1.1〜2.3</td>
</tr>
<tr>
<td>Hyaluronic acid (ng/mL)</td>
<td>53.1±30.1</td>
<td>34.8±16.5</td>
<td>&lt;50</td>
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<tr>
<td>IgG (mg/dL)</td>
<td>1166±229</td>
<td>1135±231</td>
<td>870〜1700</td>
</tr>
<tr>
<td>IgM (mg/dL)</td>
<td>79.4±24.4</td>
<td>79.7±26.8</td>
<td>33〜190</td>
</tr>
<tr>
<td>MMP-3 (ng/mL)</td>
<td>88.5±23.0</td>
<td>91.2±22.1</td>
<td>36.9〜121</td>
</tr>
<tr>
<td>Platelet (×10^4 /μL)</td>
<td>23.4±4.7</td>
<td>19.5±5.9</td>
<td>13.1〜36.2</td>
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<td>Erythrocyte sedimentation rate , 1h (mm)</td>
<td>4.9±2.3</td>
<td>5.1±2.4</td>
<td>1〜7</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate , 2h (mm)</td>
<td>11.7±6.2</td>
<td>13.3±6.8</td>
<td></td>
</tr>
<tr>
<td>CRP (ng/mL)</td>
<td>927±1369</td>
<td>802±837</td>
<td>&lt;1500</td>
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</table>

Mean±S.D. (n=9)
Fig. 14. The effect of Red Ginger Extract (oral) on blood hyaluronic acid
5. Stability of RED GINGER EXTACT
(1) Thermostability
Evaluation on the thermostability of Red Ginger Extract (without binder) was conducted at 80°C & 100°C for 1 hour. As shown in Fig. 15, degradation of [6]-gingerol begins upon heating at 100°C due to decomposition of its essential oil components. Meanwhile, tannin degraded at 80°C and increased again at 100°C along with colour change (reddish brown) due to probable polymerization.

![Fig. 15. Thermostability of RED GINGER EXTRACT](image1)

Changes on components of Red Ginger Extract in aqueous condition under commercial standard was studied. Red Ginger Extract-P 0.01% solution was prepared and heated according to the condition stipulated for sterilization of bottled beverages (i.e. 15 min at 85°C or 121°C). Results showed that content of [6]-gingerol and tannin were highly stable at high temperature.

![Fig. 16. Thermostability of RED GINGER EXTRACT-P in sterilizing condition](image2)
(2) pH stability
Evaluation on the pH stability of Red Ginger Extract was conducted. 0.01% and 0.1% of Red Ginger Extract-P solution was prepared and stored at different pH at 4°C for 3 days under darkness. Results showed that [6]-gingerol and tannin are highly stable ranges of pH 3 to pH 8.

![Fig. 17. pH stability of RED GINGER EXTRACT-P](image)

(3) Photostability
Photostability of Red Ginger Extract was carried out using Red Ginger Extract-LC (containing Red Ginger Extract 1%) and stored under fluorescent light for 1 month. Results showed that there was no degradation of [6]-gingerol under fluorescent light.

![Fig. 18. Photostability of RED GINGER EXTRACT-LC](image)
(4) Preservation stability

Study on the stability of Red Ginger Extract (without diluent) under preserved condition at room temperature started 8 months ago and still in progress. No significant degradation in [6]-gingerol observed to-date.

![Graph showing stability of preserved RED GINGER EXTRACT (without diluent)](image)

**Fig. 19. Stability of preserved RED GINGER EXTRACT (without diluent)**

### 6. Nutrition information (RED GINGER EXTRACT)

<table>
<thead>
<tr>
<th>Description</th>
<th>RED GINGER EXTRACT-P</th>
<th>RED GINGER EXTRACT-WSP</th>
<th>Note</th>
<th>Analytical method</th>
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<tr>
<td>Water</td>
<td>5.4 g/100g</td>
<td>5.4 g/100g</td>
<td></td>
<td>Distillation method</td>
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<tr>
<td>Protein</td>
<td>37.4 g/100g</td>
<td>24.9 g/100g</td>
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<tr>
<td>Fat</td>
<td>2.0 g/100g</td>
<td>1.3 g/100g</td>
<td></td>
<td>Soxhlet extraction method</td>
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<tr>
<td>Ash</td>
<td>16.6 g/100g</td>
<td>11.1 g/100g</td>
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<td>Direct incineration</td>
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<tr>
<td>Carbohydrate</td>
<td>32.9 g/100g</td>
<td>53.5 g/100g</td>
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<tr>
<td>Energy</td>
<td>311 kcal/100g</td>
<td>333 kcal/100g</td>
<td>3</td>
<td>Revised Atwater method</td>
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<tr>
<td>Dietary fiber</td>
<td>5.7 g/100g</td>
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<td></td>
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<td>Sodium</td>
<td>120 mg/100g</td>
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<td></td>
<td>Atomic absorption spectrophotometry</td>
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1. Nitrogen, protein, conversion factor: 6.25
2. Calculation : 100 – (water+protein+fat+ash+dietary fiver)
3. Energy expression standard : protein 4, fat 9, sugar 4, dietary fiver 2

Test trustee : Farco Biosystem Co. Ltd
Date of analysis : April 26, 2006
Test No. REP03540
7. Safety of RED GINGER EXTRACT

(1) Residual agricultural chemicals

Red Ginger Extract (without binder) and Red Ginger Oil are compliant to the standards stipulated in the Food Sanitation Law by the Ministry of Health, Labour & Welfare for the 33 residual agricultural chemicals. None of the Residual Agricultural Chemicals is detected.

<table>
<thead>
<tr>
<th>Description</th>
<th>Result</th>
<th>Detection limit</th>
<th>Method</th>
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</thead>
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<tr>
<td>1 EPN</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
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<tr>
<td>2 EPTC</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>3 Acephate</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>4 Iprodione</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>5 Etofenprox</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>6 Etorimfos</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>7 Cadusafos</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>8 Chlorpyrifos</td>
<td>Not detected</td>
<td>0.05 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>9 Diethofencarb</td>
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<td>GC-MS</td>
</tr>
<tr>
<td>10 Cyhalothrin</td>
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<td>11 Thiobencarb</td>
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<td>13 Tralomethrin</td>
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<td>14 Trifural;in</td>
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<td>Not detected</td>
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<td>GC-MS</td>
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<td>16 Parathion-methyl</td>
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<td>0.05 ppm</td>
<td>GC-MS</td>
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<td>17 Biosoemthrin</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>18 Pirimisfos-methyl</td>
<td>Not detected</td>
<td>0.02 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>19 Fenarimol</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>20 Fenobucarb</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>21 Fenvalerate</td>
<td>Not detected</td>
<td>0.05 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>22 Furcythrinate</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>23 Flutolanil</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>24 Prothiofos</td>
<td>Not detected</td>
<td>0.05 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>25 Propamocarb</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>26 Permethrin</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>27 Penecuron</td>
<td>Not detected</td>
<td>0.5 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>28 Boscalid</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>29 Methiocarb</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>30 Metribuzin</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>31 Lenacil</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>32 Dichlorvos</td>
<td>Not detected</td>
<td>0.1 ppm</td>
<td>GC-MS</td>
</tr>
<tr>
<td>33 Triflumizole</td>
<td>Not detected</td>
<td>0.05 ppm</td>
<td>GC-MS</td>
</tr>
</tbody>
</table>

Test trustee: Kyusai Analytical Laboratory
Date of analysis: May 9, 2006
Test No. 2006032326-01,02

(2) Acute toxicity (LD50)

Safety of Red Ginger Extract was conducted according to the Pharmaceuticals Guidelines on Single-dose Toxicity Test. 2000mg/kg of Red Ginger Extract (maximum dosage without burden on animals) was given to fasting ICR male/female mice of 5-week old followed by close observation for 14 days. No fatal event and no abnormal changes observed in the weight of mice (compared to control). Similarly, no abnormal changes detected in organs of mice upon partial inspection conducted after the test. The LD50 (oral) of Red Ginger Extract on male/female mice is deduced to be >2,000mg/kg.
(3) **Four-week repeated dose toxicity test**
Toxicity on 28-day repeated dose was conducted on SD male rats. 50, 150 & 500mg/kg of Red Ginger Extract was given to rats without any restrictions on weight and test condition during the 28-day period. No abnormal changes observed in organs, weight and blood profile of rats at end of test.

(4) **Mutagenicity**
Ames Test was conducted and finding was Negative. Red Ginger Extract is non-mutagenic.

8. **Recommended Dose of Red Ginger Extract**
Recommended daily dose: 15 to 20 mg of RED GINGER EXTRACT-P.

9. **Crude Drug Equivalent**
1g of RED GINGER EXTRACT-P is equivalent to approximately 44 g of raw red ginger. Recommended daily dose is equivalent to 660 to 880 g of raw red ginger.

10. **Commercial application**

<table>
<thead>
<tr>
<th>Application</th>
<th>Claims</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foods</td>
<td>Relief of joint pain</td>
<td>Prevention of arthropathy (knee arthritis, rheumatism, etc)</td>
</tr>
<tr>
<td>Cosmetics</td>
<td>Anti-aging cosmetics</td>
<td>Body lotion, body gel, etc</td>
</tr>
</tbody>
</table>

11. **Packaging**
RED GINGER EXTRACT-P (powder, for food), -WSP (water-soluble powder, for food)
5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard
RED GINGER EXTRACT-PC (powder, for cosmetics), -WSPC (water-soluble powder, for cosmetics)
5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard
RED GINGER EXTRACT –LC (water-soluble liquid, for cosmetics)
5kg Interior packaging: aluminium bag
Exterior packaging: Cardboard

12. **Storage**
Store in cool, dry dark place.

13. **Expression**
<Food>
Red ginger extract-P , -WSP
PRODUCT STANDARD

PRODUCT NAME

RED GINGER EXTRACT - P

Food

This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous ethanol. It guarantees minimum 6.0% [6]-gingerol and 1.5% tannin. A peak of 3R,5S-[6]-gingerdiol is observed in HPLC chromatogram.

**Appearance**
Light yellow or yellowish brown powder with unique smell and pungent flavor.

**[6]-gingerol and [6]-shogaol**
Min. 6.0% (HPLC)

**Tannin**
Min. 1.5% (Vanillin • HCl method) (Equivalent of procyanidin B2)

**3R,5S-[6]-gingerdiol**
Detectable peak (HPLC)

**Loss on drying**
Max. 10.0% (Analysis for Hygienic Chemists, 1 g, 105°C, 2 h)

**Purity test**

(1) Heavy metals
Max. 30 ppm (The Japanese Standards for Food Additives)

(2) Arsenic
Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

**Standard Plate Counts**
Max. 3 x 10³ cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. 1 x 10³ cfu/g (Analysis for Hygienic Chemists)

**Coliforms**
Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red ginger extract</td>
<td>50%</td>
</tr>
<tr>
<td>Cyclodextrin</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
</tr>
</tbody>
</table>
This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous ethanol. It guarantees minimum 3.0% [6]-gingerol and 0.5% tannin. A peak of 3R,5S-[6]-gingerdial is observed in HPLC chromatogram.

**Appearance**

Yellowish white powder with slightly unique smell and pungent flavor.

**[6]-gingerol and [6]-shogaol**

Min. 3.0 % (HPLC)

**Tannin**

Min. 0.5 % (Vanillin • HCl method)

(Equivalent of procyanidin B2)

**3R,5S-[6]-gingerdial**

Detectable peak (HPLC)

**Loss on drying**

Max. 10.0 % (Analysis for Hygienic Chemists, 1 g, 105 °C, 2 h)

**Purity test**

(1) Heavy metals

Max. 20 ppm (The Japanese Standards for Food Additives)

(2) Arsenic

Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

**Standard Plate Counts**

Max. 3 × 10³ cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**

Max. 1 × 10³ cfu/g (Analysis for Hygienic Chemists)

**Coliforms**

Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red ginger extract</td>
<td>33 %</td>
</tr>
<tr>
<td>Cyclodextrin</td>
<td>67 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
</tr>
</tbody>
</table>
This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous ethanol. It guarantees minimum 6.0% [6]-gingerol and 1.5% tannin. A peak of 3R,5S-[6]-gingerdial is observed in HPLC chromatogram.

**Appearance**
Light yellow or yellowish brown powder with unique smell and pungent flavor.

**[6]-gingerol and [6]shogaol**
Min. 6.0 % (HPLC)

**Tannin**
Min. 1.5 % (Vanillin • HCl method)

(Equivalent of procyanidin B₂)

**3R,5S-[6]-gingerdial**
Detectable (HPLC)

**Loss on drying**
Max. 10.0 % (1 g, 105 °C, 2 h)

**Purity test**
(1) Heavy metals
Max. 30 ppm (The second method)

(2) Arsenic
Max. 1 ppm (The third method)

**Standard Plate Counts**
Max. 1 × 10² cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. 1 × 10² cfu/g (Analysis for Hygienic Chemists)

**Coliforms**
Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red ginger extract</td>
<td>50 %</td>
</tr>
<tr>
<td>Cyclodextrin</td>
<td>50 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
</tr>
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</table>

Ref: The Japanese Standards of Quasi-Drug Ingredients.
PRODUCT STANDARD

PRODUCT NAME

RED GINGER EXTRACT - WSPC
Cosmetics

This product is extracted from red ginger, the rhizome of Zingiber officinale var. Rubra, with aqueous ethanol. It guarantees minimum 3.0% [6]-gingerol and 0.5% tannin. A peak of 3R,5S-[6]-gingerdial is observed in HPLC chromatogram.

**Appearance**
Yellowish white powder with slightly unique smell and pungent flavor.

**[6]-gingerol and [6]-shogaol**
Min. 3.0 %  
(HPLC)

**Tannin**
Min. 0.5 %  
(Vanillin • HCl method)
(Equivalent of procyanidin B2)

**3R,5S-[6]-gingerdial**
Detectable peak  
(HPLC)

**Loss on drying**
Max. 10.0 %  
(1 g, 105 °C, 2 h)

**Purity test**
( 1 ) Heavy metals
Max. 20 ppm  
(The second method)

( 2 ) Arsenic
Max. 1 ppm  
(The third method)

**Standard Plate Counts**
Max. 1 × 10^3 cfu/g  
(Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. 1 × 10^2 cfu/g  
(Analysis for Hygienic Chemists)

**Coliforms**
Negative  
(Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclodextrin</td>
<td>67 %</td>
</tr>
<tr>
<td>Red ginger extract</td>
<td>33 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Ref: The Japanese Standards of Quasi-Drug Ingredients.
PRODUCT STANDARD

PRODUCT NAME

RED GINGER EXTRACT-LC

Cosmetics

This product is extracted from red ginger, the rhizome of *Zingiber officinale* var. Rubra, with aqueous 1,3-butylene glycol. It guarantees minimum 0.1% [6]-gingerol and 0.01% tannin. A peak of 3R,5S-[6]-gingerdial is observed in HPLC chromatogram.

**Appearance**
Orange or blown liquid with unique smell.

**[6]-gingerol**
Min. 0.1 % (HPLC)

**Tannin**
Min. 0.01 % (Vanillin • HCl method)

(Equivalent of procyanidin B₂)

**3R,5S-[6]-gingerdial**
Detectable peak (HPLC)

**Purity test**

(1) **Heavy metals**
Max. 10 ppm (The second method)

(2) **Arsenic**
Max. 1 ppm (The third method)

**Standard Plate Counts**
Max. $1 \times 10^2$ cfu/g (Analysis for Hygienic Chemists)

**Moulds and Yeasts**
Max. $1 \times 10^2$ cfu/g (Analysis for Hygienic Chemists)

**Coliforms**
Negative (Analysis for Hygienic Chemists)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butylene glycol</td>
<td>70 %</td>
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<tr>
<td>Water</td>
<td>29 %</td>
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<tr>
<td>Red ginger extract</td>
<td>1 %</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100 %</td>
</tr>
</tbody>
</table>

Ref: The Japanese Standards of Cosmetic Ingredients.
ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact:

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493-8001 JAPAN
TEL : +81 (0) 586 86 5141
FAX : +81 (0) 586 86 6191
URL/http : //www.oryza.co.jp/
E-mail : info@oryza.co.jp

Revised points:
Product standard (P, WSP, PC, WSPC)
- [6]-gingerol □ [6]-gingerol+[6]-shogaol
- Heavy metal 10 □ 30 ppm (P, PC), 10 □ 20 ppm (WSP, WSPC)

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Revised Date: December 3, 2007