

# cosmeHerbest™ BLACK CUMIN

This product is an extract obtained from the seeds of *Nigella sativa* Linné (*Ranunculaceae*) by supercritical extraction method, it comprises 1.5 to 4.5% of Thymoquinone ( $C_{10}H_{12}O_2$  : 164.2).

## Manufacturing method

Black Cumin Extract is obtained from the seeds of *Nigella sativa* Linné (*Ranunculaceae*) by supercritical extraction method. Separately, Rosemary Leaf Extract is obtained from the leaves of *Rosmarinus officinalis* Linné (*Lamiaceae*) by supercritical extraction method. Mix 0.1% of Rosemary Leaf Extract into 99.9% of Black Cumin Extract as the product.

Raw material: Seeds of *Nigella sativa* Linné 5-6 kgs  $\longrightarrow$  Product: about 1.0 kg

## Description

This product is a brown to slightly yellowish brown oily liquid, and it is having characteristic odor.

## Identification

- Infrared Spectrophotometry

Determine the infrared absorption spectra of this product as directed liquid film method; exhibit wavelength at around  $2926\text{cm}^{-1}$ ,  $1745\text{cm}^{-1}$ ,  $1458\text{cm}^{-1}$ .

- Thymoquinone

To 1 mL of this product, add 2 mL of methanol, shake well, centrifuge and stand to allow for 20 minutes. After standing, a supernatant methanol layer is used as the test solution. Separately, dissolve 10mg of Thymoquinone RS (for example, manufactured by Tokyo Kasei Kogyo Co., Ltd.) in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography. Spot of  $5\mu\text{L}$  of the test solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and isopropyl ether (198 : 2) and air-dry the plate. One spot each of the test solution and the standard solution show the same Rf value at around 0.8.

**Specific Gravity**  $d_{20}^{20}$  : 0.915 to 0.925 (Method 1, C)

## Purity Test

- Heavy metals

Take 2.0 g of this product, determine heavy metals according to method 2: the limit is not more than 10 ppm. Use 2.0 mL of standard lead solution as the control solution.

- Arsenic

Take 2.0 g of this product, prepare the test solution according to method 3, and perform the test: the limit is not more than 1 ppm.

**Residue on Ignition** : 0.5 % max. (1g,  $550^{\circ}\text{C}$ , Method 2)

**Moisture** : 1.0 % max. (25mL, Volumetric titration method, Direct titration)

50 mL of a dehydrating solvent CM (manufactured by Mitsubishi Kasei Kogyo Co., Ltd.) which has been attached to a titration device in advance is placed in a titration flask and dropped to make it anhydrous by dripping a Karl Fischer reagent SS (manufactured by Mitsubishi Kasei Kogyo Co., Ltd.) The amount of Karl Fischer reagent SS consumed in this case need not be read). Add 25 mL of this product correctly, stir at room temperature for about 15 minutes, completely transfer the moisture to the dehydrated solvent CM, titrate with Karl Fischer reagent SS (titer F mg H<sub>2</sub>O / mL) and calculate its consumption Calculate moisture W from quantity A mL.

$$W (\%) = A \times F \times 100 / (25 \times \text{value of specific gravity test} \times 1000)$$

**Assay** <Reference Analysis>

- Thymoquinone

Weigh accurately about 100mg of this product, dissolve by adding 2 mL of tetrahydrofuran, and furthermore add acetonitrile exactly to 20 mL (C<sub>T</sub>), filter, and use the filtrate as the test solution. Separately weigh accurately about 2 mg of Thymoquinone RS (for example, manufactured by Tokyo Kasei Kogyo Co., Ltd.) and dissolve it in a mixture of tetrahydrofuran: acetonitrile = 1: 9 to make exactly 10 mL (C<sub>S</sub>), and use it as the standard solution. Take exactly 10 μL each of the test solution and the standard solution as directed under the Liquid-chromatography in accordance with the following conditions, and measure the peak areas A<sub>T</sub> and A<sub>S</sub> of the Thymoquinone of each solution.

The Content (%) of Thymoquinone ( C<sub>10</sub>H<sub>12</sub>O<sub>2</sub> : 164.2) = (A<sub>T</sub> × C<sub>S</sub>) / (A<sub>S</sub> × C<sub>T</sub>) × 100

A<sub>T</sub>: The area of thymoquinone in the test solution

A<sub>S</sub>: The area of thymoquinone in the standard solution

C<sub>T</sub>: Collection amount (mg) / Adjustment amount (mL) of the test solution

C<sub>S</sub>: Collection amount (mg) / Adjustment amount (mL) of the standard solution

*Operating Conditions:-*

Detector : An ultraviolet absorption photometer (wavelength: 254nm)

Column : A stainless steel column 4.6 mm in inside diameter and 25cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5μm in particle diameter).

Column Temperature: A constant temperature of about 35°C.

Mobile Phase : A mixture of water and methanol (45:55)

Flow rate : 1.3mL (Adjust a flow rate so that the retention time of Thymoquinone is about 10 minutes.)

**Bacterial Count**

Take 5g of this solution, make 50mL test solution with diluent and perform the bacterial count test, using standard agar medium according to Hygiene Test Method; the limit is not more than  $1 \times 10^2$  cfu/g.

**Fungus Count**

Take 5g of this solution, make 50mL test solution with diluent and perform the fungus count test using potato dextrose agar medium added chloramphenicol according to Hygiene Test Method; the limit is not more than  $1 \times 10^2$  cfu/g.

## Coliforms

Take 1mL of the solution which prepare the bacterial count test, and perform the coliform test using BGLB medium according to Hygiene Test Method; Negative / Not observe any colony.

These standards and test method are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

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| Product Name | : cosmeHerbest™ BLACK CUMIN   |
| Expiry date  | : 2 years from date of manufacturing  |
| Storage      | : Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container. |
| Manufacturer | : ORYZA OIL & FAT CHEMICAL CO., LTD.<br>1 Aza Numata Kitagata Kitagata-cho Ichinomiya-city,<br>Aichi-pref. 493-8001 JAPAN                       |

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