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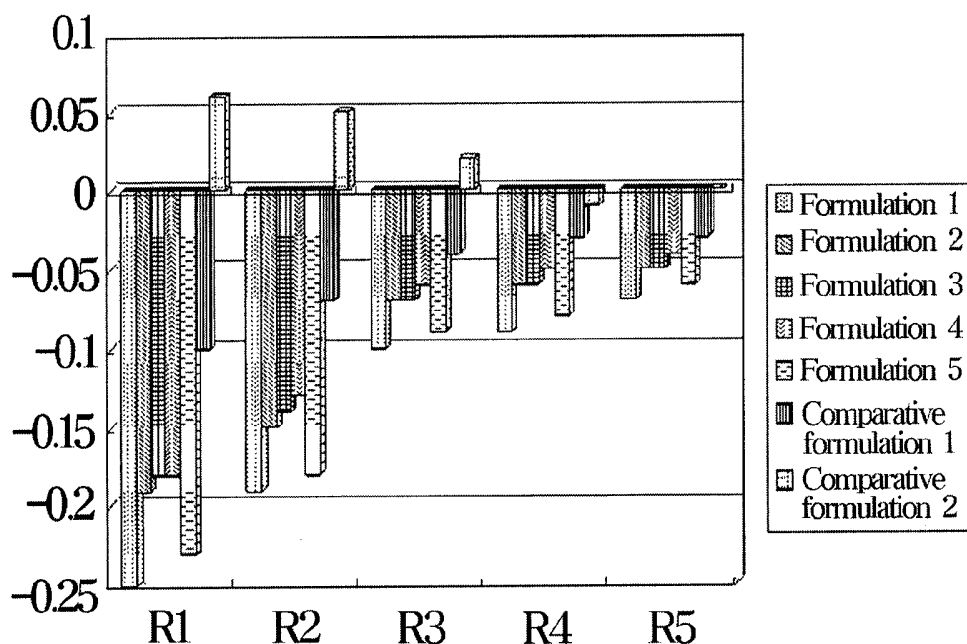
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[Continued on next page]

(54) Title: ANTI-AGING COSMETIC COMPOSITION

(57) Abstract: The present invention relates to an anti-aging cosmetic composition, and more particularly, the present invention relates to an anti-aging cosmetic composition containing *Hibiscus esculentus* extracts and at least one selected from the group consisting of oleanolic acid, ursolic acid, glycyrrhetic acid and retinol, showing high safety to skin and wrinkle-improvement effect.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

ANTI-AGING COSMETIC COMPOSITION

Technical Field

The present invention relates to an anti-aging cosmetic composition,
5 and more particularly to an anti-aging cosmetic composition showing superior
safety to skin and skin-wrinkle improvement, containing Hibiscus esculentus
extracts and at least one selected from the group consisting of oleanolic acid,
ursolic acid, glycyrrhetic acid and retinol.

10 **Background Art**

Human skin, as a primary protective barrier, protects the vital organs of
the body from external irritants such as changes in temperature and humidity,
ultraviolet rays and contaminants, and plays an important role in the
regulation of biological homeostasis such as thermoregulation. However, as
15 skin ages, it shows skin aging effects such as loss of elasticity, keratinization,
formation of skin wrinkles and skin contraction. The cause of this skin aging
can be classified as internal factors such as cell gene transformation and cell
tissue change, and external factors such as UV and humidity (Masamitsu
Ichihashi, Fragrance Journal, Vol. 32, No. 5, 24-30). In particular, skin
20 aging effect due to UV is called "photoaging". In photoaging, oxygen free
radicals are generated in cells by UV light, and the oxygen free radicals
accelerate the synthesis of protease (MMP-1, MMP-3, MMP-9, etc.), enzyme
that decomposes protein such as collagen or elastin that is elasticity fiber of
derma, by way of the signal transduction system raising an inflammation
25 reaction, and thereby decreasing the elasticity of derma and producing the skin
wrinkle.

Therefore, in order to prevent skin aging and keep more healthy and elastic skin, studies have been made to develop physiological active substances having a skin wrinkle improvement effect. For example, in 1995, trans-retinoic acid was approved by FDA as a therapeutic for the improvement of photoaged skin. In addition, retinol, which has fewer side effects, has been used in cosmetics since the mid 1990's, and various cosmetics for skin wrinkle improvement have appeared on the market. Since then, female hormonally active agents or antioxidants extracted from various plants have been introduced to cosmetics as skin wrinkle improving agents.

However, most of the above agents have many problems such as weak efficacy or serious side effects. Thus, extensive studies have been undertaken on substances having an anti-aging effect with no side effects. Among these, there has been much attention on peptides, which have various biological activities, as novel ingredients of highly functional cosmetics. Peptides are biopolymer having 20 essential amino acids linked via amide bond, and it has been expected that peptides would produce direct effects on *in vivo* metabolism due to their great interaction with protein.

Korean Laid-Open Publication No. 2001-0006358 discloses a cosmetic composition containing protein fraction extracted from the seed of Hibiscus esculentus. Examples are disclosed regarding a process for extracting protein fractions from Hibiscus esculentus and a cosmetic composition containing the protein fraction; however, no use is disclosed of the cosmetic composition containing extracts of Hibiscus esculentus for anti-aging.

Recently, much attention has been paid to the treatment of "expression wrinkle," which is generated by mechanical movement of facial muscle, as well as to the treatment of endogenous aging generated by aging and of

photoaging generated by UV light. With regard to the improvement of expression wrinkle, botulic toxin (Botox), which inhibits acetylcholine secretion in neuron tissue and thereby paralyzes muscle contraction, has been used. However, injection by doctors is required due to its toxicity, and it is prohibited from being used as in cosmetics. Therefore, extensive studies have been made on cosmetics derived from natural plants that can replace Botox and guarantee safety to skin.

Therefore, the present inventors have studied the development of cosmetic materials derived from natural plants not inducing any side effect to skin and having excellent effects in skin aging prevention and skin wrinkle improvement, and have found that a skin-aging cosmetic composition having excellent effects in skin aging prevention and skin wrinkle improvement can be made by containing Hibiscus esculentus extracts, which improve skin wrinkles by inhibiting muscle contraction and removing oxygen free radicals, and active ingredients selected from oleanolic acid, ursolic acid, glycyrrhetic acid and retinol, which improve skin wrinkles generated by aging.

The Hibiscus esculentus extracts can comprise oligopeptides extracted from Hibiscus esculentus and processed, as active ingredients.

The object of the present invention is to provide an anti-aging cosmetic composition having excellent skin safety and excellent effects in skin aging prevention and skin wrinkle improvement.

Disclosure of Invention

To attain the above object, there is provided an anti-aging cosmetic composition containing Hibiscus esculentus extracts and at least one selected

from the group consisting of oleanolic acid, ursolic acid, glycyrrhetic acid and retinol.

The *Hibiscus esculentus* extracts are obtained by methods known in the art, for example, leafs, seeds and roots of *Hibiscus esculentus*, which come
5 under the category of mellow flowers, are dried, pulverized and extracted by using water or organic solvent. The organic solvent can be polar solvent such as ethanol, butanol or acetone, or non-polar solvent such as ether, hexane, benzene, chloroform or ethyl acetate, or a mixture thereof.

The *Hibiscus esculentus* extracts may contain oligopeptides extracted
10 from *Hibiscus esculentus* and processed, as active ingredients. The oligopeptides complex according to the present invention removes oxygen free radicals that reduce the function of bio-molecules contributing to skin elasticity, and thereby protects skin from biological oxidation reaction. In addition, it can improve skin wrinkle by increasing collagen synthesis in skin,
15 which leads to an increase of elasticity fiber in the skin derma. Further, it has effects on muscle contraction inhibition or muscle relaxation. In particular, muscle relaxation effects appear in facial expression muscle, which is used when people make a facial expression such as smiling or frowning, and the oligopeptides complex can decrease contractile force of expression
20 muscle, thereby alleviating skin wrinkle temporarily produced at the time of making a facial expression, and preventing the fixation of such temporary skin wrinkle.

In addition, the oligopeptides complex is a mixture of low molecular oligopeptides whose average molecular weight is 262 Da (Dalton), and the
25 content of the oligopeptides complex is preferably 0.00001~10 wt% based on the total weight of the composition. If the content is less than 0.00001 wt%,

skin wrinkle improvement effect is insignificant, and if the content is more than 10 wt%, the efficacy does not significantly increase, thus the efficiency of the material is low.

The oleanolic acid, ursolic acid, glycyrrhetic acid and retinol are each preferably contained in an amount of 0.00001~10 wt% based on the total weight of the composition. If the content is less than 0.00001 wt%, skin wrinkle improvement effect is insignificant, and if the content is more than 10 wt%, the efficacy does not significantly increase, thus the efficiency of the material is low.

The formulation of the cosmetic composition in the present invention is not limited to a specific kind. It may be formulated into skin softeners, nutrient toilet water, massage creams, nutrient creams, gels, packs or skin-adhesive type formulations.

Brief Description of Drawings

Figure 1 is a graph showing skin wrinkle improvement of Formulation Examples 1~5 and Comparative Formulation Examples 1 and 2.

Best Mode for Carrying Out the Invention

The present invention will be described in more detail by way of the following examples. However, these examples are provided for the purpose of illustration only and should not be construed as limiting the scope of the invention, which will be apparent to one skilled in the art.

[Experimental Example 1] Effect on promoting collagen synthesis

Human fibroblast was cultured in 24-well microplates, and the culture

medium thereof was changed to one containing Hibiscus esculentus extracts at concentrations of 0, 1, 5 and 10 ppm, respectively. The Hibiscus esculentus extracts used were commercially available oligopeptides obtained from the extracts of Hibiscus esculentus, whose product name is MYOXINOL (INCI name: Hydrolyzed Hibiscus esculentus Extract: Manufactured by Laboratoires Sérobiologiques). At 3 days of cultivation, 0.5 ml of DMEM culture medium containing 10% FBS was added to each well, then 10 µCi of L[2,3,4,5-3H]-proline was added thereto. 24 hours later, the culture medium and the cells contained in each well were raked, and put into the 5% TCA (Trichloroacetic acid) solution. Each sample was then divided into 2 test tubes, and 1 unit/µl of type I collagenase was added to one of each pair of the test tubes, and cultured at 37°C for 90 minutes. The other test tube of each pair was stored at 4°C. 0.05 ml of 50% TCA was then added to every test tube, and the test tubes were left at 4°C for 20 minutes, then each was centrifuged at 12,000 rpm for 10 minutes. Each supernatant and sediment was treated with Liquid Scintillation Counter (LSC) to obtain a CPM (Count Per Minute) value, then Relative Collagen Biosynthesis value (RCB) of control and experimental group was obtained based on the below Numerical Formula 1. The results are shown in Table 1.

20

[Numerical Formula 1]

$$\text{RCB (\%)} = (\text{cpm of collagen}) / \{(\text{cpm of total collagen}) \times 5.4 + \text{cpm of collagen}\} \times 100$$

[Table 1]

Concentration of Hibiscus esculentus extracts (ppm)	Relative collagen biosynthesis value (RCB) (%)
--	---

10	140
5	122
1	112
0 (Control)	100

As can be seen in the above Table 1, Hibiscus esculentus Extracts increased the collagen biosynthesis value of fibroblast in a concentration-dependent manner.

5

[Experimental Example 2] Effect on muscle relaxation

Sprague-Dawley rats were bled to death, then their diaphragm and phrenic nerves were excised. The tissue slices thereof were put into organ chambers, and the chambers were linked to a force displacement transducer (Grass Co.). 2 kg of tension was applied thereto and electrostimulation was applied for 0.2 msec at intervals of 20 sec at a voltage by which maximum contraction can be shown. 60 minutes later, whether the contraction of diaphragm was constant was checked, and 0, 50, 500, 1000 and 2000 ppm of Hibiscus esculentus Extracts were added to the organ chambers to estimate the effect on inhibiting the contraction of diaphragm. The Hibiscus esculentus extracts used were commercially available oligopeptides obtained from the extracts of Hibiscus esculentus, whose product name is MYOXINOL (INCI name: Hydrolyzed Hibiscus esculentus Extract: Manufactured by Laboratoires Sérobiologiques).

20

[Table 2]

Concentration of Hibiscus esculentus extracts (ppm)	Ratio of inhibiting diaphragm contraction (%)
--	--

0 (control)	0
50	4.0
500	17.9
1000	21.8
2000	30.7

As can be seen in the above Table 2, Hibiscus esculentus Extracts according to the present invention inhibited diaphragm contraction in a concentration-dependent manner, which means that Hibiscus esculentus Extracts have muscle relaxation effects.

Formulation Examples 1-5 and Comparative Formulation Examples 1-2]

To estimate the skin wrinkle alleviation effect, cream formulations were prepared in the following compositions shown in Table 3 by a conventional method.

[Table 3]

[illegible]

Butylene Glycol	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vegetable Hydrogenated Oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Stearic acid	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Glyceryl stearate	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cetearyl alcohol	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Arachidyl/Behenyl Alcohol & arachidyl glucoside	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cetyl aryl alcohol & cetearyl glucoside	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Liquid paraffin	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Caprylic/carlic triglyceride	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Carbomer	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Triethanol amine (TEA)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Preservative, Perfume, pigment	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	To100	To100	To100	To100	To100	To100	To100

The Hibiscus esculentus extracts used were commercially available oligopeptides obtained from the extracts of Hibiscus esculentus, whose product name is MYOXINOL (INCI name: Hydrolyzed Hibiscus esculentus Extract: Manufactured by Laboratoires Sérobiologiques).

[Experimental Example 3] Improvement of skin wrinkle

The following was performed to identify the effects of the above formulations in Table 3 on the improvement of skin wrinkle. 140 women aged 30-39 were divided into 7 groups (20 people per group). To each group, one of the formulations 1, 2, 3, 4, 5, comparative formulations 1 and 2 was

applied twice per day for 8 weeks, and replicas were prepared after 8 weeks using silicon. The state of skin wrinkles was image analyzed by visiometer (SV600, Courage+Khazaka Electronic GmbH, Germany). The average of the values obtained by subtracting each parameter value before the application from the same person's corresponding parameter value 8 weeks later are shown in Table 4.

【Table 4】

Clinical result 8 weeks after use	R1	R2	R3	R4	R5
Formulation 1	-0.25	-0.19	-0.1	-0.09	-0.07
Formulation 2	-0.19	-0.16	-0.07	-0.06	-0.05
Formulation 3	-0.18	-0.14	-0.07	-0.06	-0.05
Formulation 4	-0.18	-0.13	-0.06	-0.05	-0.04
Formulation 5	-0.23	-0.18	-0.09	-0.08	-0.06
Comparative Formulation 1	-0.10	-0.07	-0.04	-0.03	-0.03
Comparative Formulation 2	0.06	0.05	0.02	-0.01	0.00

R1: Difference between the maximum value and the minimum value of skin wrinkle contour

R2: Average of R1 obtained by the difference between the value of an arbitrary contour and the value of the fifth contour from the arbitrary contour

R3: Maximum of R1 obtained by the difference between the value of an arbitrary contour and the value of the fifth contour from the arbitrary contour

R4: Average of each peak-to-peak value at the baseline of wrinkle contour

R5: Difference between the baseline and the value of each wrinkle contour

As can be seen in Table 4 and Fig. 1, the formulations 1~5, which contain Hibiscus esculentus extracts and at least one selected from the group consisting of oleanolic acid, ursolic acid, glycyrrhetic acid and retinol, are superior in improvement of skin wrinkle to the comparative formulations 1 and 2, and especially the effect of formulation 1 was excellent.

In addition, from the safety test of the above formulations 1~5 and the comparative formulations 1~2, it was recognized that the cosmetic composition of the present invention shows excellent safety.

Industrial Application of the Invention

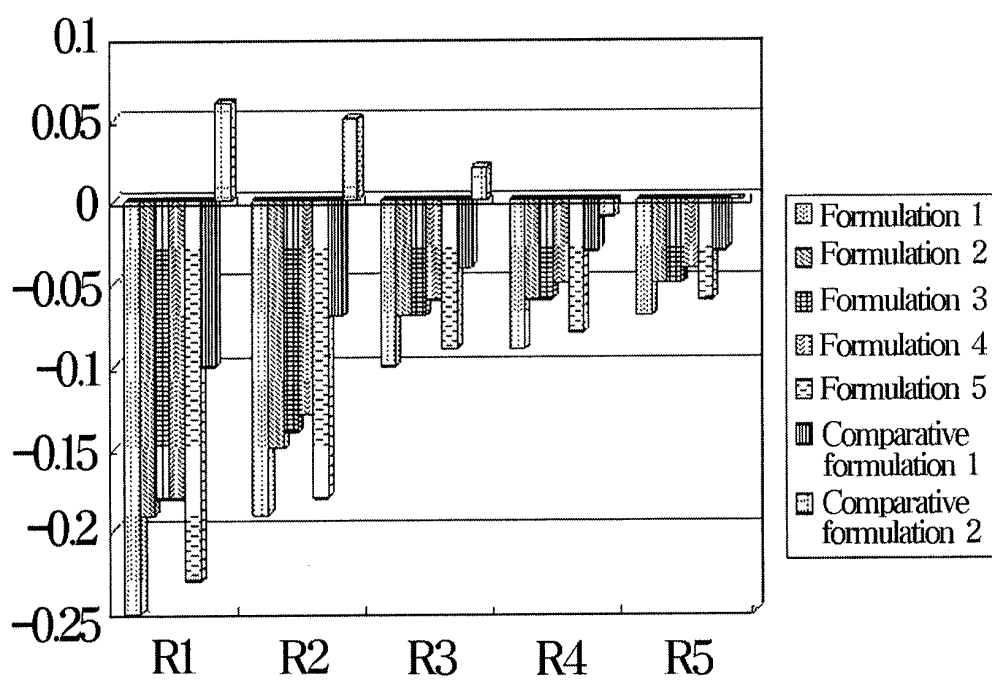
As above described, the cosmetic composition according to the present invention shows excellent safety to skin and skin wrinkle improvement. Therefore, it can be used in a composition for external application having anti-skin aging efficacy.

CLAIMS

1. A cosmetic composition containing Hibiscus esculentus extracts and at least one selected from the group consisting of oleanolic acid, ursolic acid,
5 glycyrrhetic acid and retinol.
2. The composition according to Claim 1, wherein the Hibiscus esculentus extracts contain oligopeptides extracted from Hibiscus esculentus.
- 10 3. The composition according to Claim 1 or 2, wherein said composition is for anti-aging.
4. The composition according to Claim 1 or 2, wherein said composition is for improving skin wrinkles.
- 15 5. The composition according to Claim 1 or 2, wherein said composition is for promoting muscle relaxation.
6. The composition according to Claim 1 or 2, wherein the Hibiscus
20 esculentus extracts are contained in an amount of 0.00001~10 wt% based on the total weight of the composition.
7. The composition according to Claim 1 or 2, wherein the at least one
25 selected from the group consisting of oleanolic acid, ursolic acid, glycyrrhetic acid and retinol is contained in an amount of 0.00001~10 wt% based on the total weight of the composition.

1/1

FIG. 1



PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PCT-050131	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/KR2005/003770	International filing date (<i>day/month/year</i>) 08 NOVEMBER 2005 (08.11.2005)	(Earliest) Priority Date (<i>day/month/year</i>) 31 JANUARY 2005 (31.01.2005)
Applicant AMOREPACIFIC CORPORATION et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☐ **Unity of invention is lacking** (See Box No. III)

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

- b. ☐ none of the figure is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/003770**A. CLASSIFICATION OF SUBJECT MATTER****A61Q 19/00(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61Q 19/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NCBI pubmed database, Delphion Research Intellectual Property network database,**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Liu CL et al 'In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity.' In 'Food Chem Toxicol. 2002 May, Vol.40, No.5. p635-41 see the whole document	1-7
A	Herrera-Arellano A al. 'Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial.' In Phytomedicine. 2004 Jul Vol.11, No.5. pp375-82 see the whole document	1-7
A	Sharma S al. 'Effect of Hibiscus rosa sinensis extract on hyperproliferation and oxidative damage caused by benzoyl peroxide and ultraviolet radiations in mouse skin' In Basic Clin Pharmacol Toxicol 2004 Nov Vol.95, No.5. pp220-5 see the whole document	1-7

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

06 MARCH 2006 (06.03.2006)

Date of mailing of the international search report

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