OUGON Liquid

(Scutellaria Baicalensis Extract)

ICHIMARU PHARCOS CO., LTD.

318-1 Asagi, Motosu-shi, Gifu 501-0475 JAPAN

Phone : (81) 58 320－1032

Fax : (81) 58 320－1039

http://www.ichimaru.co.jp

Revised on February 8, 2000

Revised on December 22, 2005

C O N T E N T S

1. Introduction

1-1 Utilization of Scutellaria for Cosmetics Original Plant

Scutellaria root: Scutellaria is a root of *Scutellaria baicalensis* Georgi of *Labiatae*, a perennial plant that grows mainly in China, Siberia, and the Korean peninsula. It has violet flowers of lip shape seen during summer; it is planted in Japan mainly for appreciation.

1-2 Efficacy

The dried root of Scutellaria Baicalensis Georgi is called " Ougon " as Chinese medicine it is used for its anti-inflammatory and anti-pyretic actions and as treatment for abdominal pain, vomiting, diarrhea. Ougon has been adopted by the Japanese Pharmacopoeia. The English name is *Scutellaria baicalensis* Georgi (Scutellaria Radix).

1-3 Main Components

Representative ingredients of Ougon are flavonoids such as Wogonin, Baicalin, Baicalein, Oroxylin-A. Baicalin is hydrolized to from baiclin and glucuronic acid.

2. Pharmacological Action

It is reported that the pharmacological actions of Ougon are as follows: - acceleration of cholate secretion by baicalin and baicalein together with detoxication, inhibition of capillary permeability, an anti-acetylcholine action, and as an anti-anaphylaxic action. Studies reported in, particularly by Eta, suggest the possibility of utilization as anti-allergic agent.

2-1 Utilization as Cosmetic

As to the cases of application of Ougon in regards to esthetic purpose and its related problems; In Europe the decoction of Ougon is used as diuretics and as an anti-inflammatory agent. There are also some reports of it being used to reduce swelling, edema, pain of eye, lacrimation as well as gargles for stomatitis and tonsillitis.

On the other hand, in Japan, the study results by Eta are suggestive of the possibility of using for chronic eczema. Chinese or Japanese prescriptions have few examples of single use of Ougon but the literature by Nanba describes as follows: Combination of Ougon and Saiko prevents affects of the cold and windy weather, cleans the surface of skin to make it clear. Accordingly one of the main purposes of cosmetic use has been to investigate prescription for the effect of skin care with the extract containing mainly baicaline and baicalein. Especially, it may be said that Ougon is one of the most not able substances for cosmetics for allergic persons.

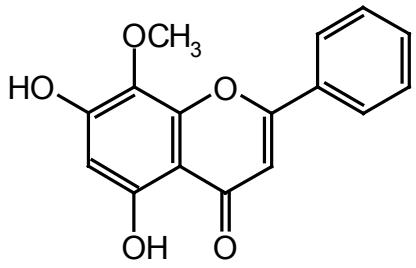
2-2 Ougon Extract for Cosmetic Ingredient

The most appropriate expression on the expected effect is "cleaning the skin surface and efficacy to make clear the skin" recorded in "Rishizai" of Chinese medical prescription kept from early days in China. Investigation on the literatures in Japan using Ougon as cosmetics shows that the extract of Ougon is listed in CTFA dictionary as " Scutellaria Root Extract ", also in Japanese Cosmetic Ingredient Dictionary JCID "V-34".

On the other hand, Ougon may be used as a cosmetic in the form of crushed powder as the handiest means or mixed with a cream base and also the decoction of it is possible to combine with other ingredients. Besides, Ougon may be used like wise as "Shiunko", by warming and immersing in various oils or may be extracted with ethanol for further use. Here a few considerations on favorable utilization of Ougon as an ingredient of cosmetic revel ate the importance of association of the ingredient contained in Ougon with its pharmacological actions.

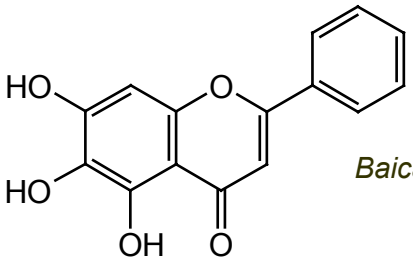
That is to say, the importance of the relationship between anti-inflammatory action and anti-allergic action of baicalin and baicalein. It means that the anti-inflammatory action is deeply related with the content of flavonoid, which seems the important point for combination with other cosmetic materials. It has been known that Ougon contains a few flavonoids (flavonols) such as woogonin, which are favorable as the component of extract for the cosmetic with respect to beauty care.

On the other hand, in extracting flavonoids, they are sparingly soluble in water but are soluble in alkaline solutions of nearly pH = 8.6. It is also soluble in ether to a minimum extract. Consequently, taking these points into consideration, the extract that is stable also in acidic pH of the usual range of general cosmetics is preferable for the preparation of extract combined as cosmetics.



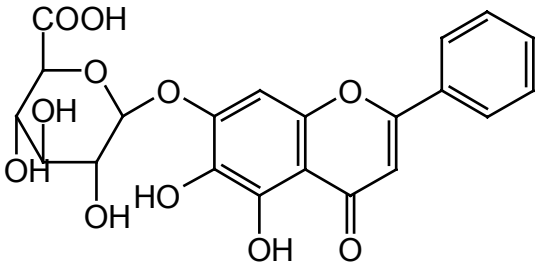
Wogonin

Molecular Weight = 284.27
Exact Mass = 284
Molecular Formula = $C_{16}H_{12}O_5$
Molecular Composition = C 67.60% H 4.23% O 28.17%



Baicalein

Molecular Weight = 270.24
Exact Mass = 270
Molecular Formula = $C_{15}H_{10}O_5$
Molecular Composition = C 66.67% H 3.73% O 29.60%



Baicalin

Molecular Weight = 446.37
Exact Mass = 446
Molecular Formula = $C_{21}H_{18}O_{11}$
Molecular Composition = C 56.50% H 4.04% O 39.46%

3 Anti-inflammatory Effect

Anti-Inflammatory Test (1)

A. Introduction

Ougon is a dried substance of the root of Scutellaria baicalensis Georgi of Labiatee, which is administered to allergic diseases by combining with Saiko drugs, such as Daisaiko-to, Shosaiko-to, Saikokeihi-to. It is mentioned that in Europe, decocuta of Ougon has been used for diuretic and anti-inflammatory drugs and also swelling, edema, pain of eye-balls, lacrimation so far as a gargle for stomatitis, tonsillitis.[1)] It is reported that the pharmacological action of Ougon is sholeretic action and the accompanied detoxication, inhibiting of capillary permeability antiacetylcholine action, antianaphylactic action.

In particular, studies by Eta suggest a possibility of utilization as anti-allergic agent. OUGON Liquid SE is a solution containing baicalein, woogonin, saccharides, amino acid, etc. extracted from the root of Scutellaria Baicalensis Georgi. The anti-inflammatory action of Ougon has been studied on edema in the paws of hind leg of rats. The detail will be reported here.

B. Experimental Materials

1. OUGON Liquid SE

OUGON Liquid SE is 70 v/v % ethanol solution and therefore, after concent-ration under reduced pressure odor disappears, it is stirred fully in amortar to make 2 w/v % suspention with purified water. Thus obtained test sample was employed.

2. Indometacin (a product of Sigma)

200 mg of indometacin was stirred fully in a mortar to suspend in 0.2 w/v % purified water. The resultant solution was used.

3. Preparation of 1 % Carragheenin Solution

On the previous day of the experiment, the solution of carragheenin (a product of Tokyo Kasei) prepared with purified water under sufficient stirring in a mortar was kept overnight in a refrigerator and used.

C. Experimental Method

Inhibition of rat paw edema produced by carragheenin in hind leg. Each of five female Wister rats weighing approximately 140 g was grouped and after measuring the volume of right hind leg, phlogogenic substance (1% carragheenin: 0.05 ml) was injected subscutaneously into the planter paw.

Then, the volume in the injected paw was measured hourly. The rate of swelling was calculated from the volume that before administration of the phlogogenic substance and the rate of inhibition were obtained by comparing with the rate of swelling in control group. The test substance was orally administered 60 minutes before admininstration of the phlogogenic.

D. Result

Time	Control		OUGON Liquid SE		Indomethacin
(hour)	Swelling (%)	Swelling (%)	Inhibitory	Swelling (%)	Inhibitor
1	28.32 ± 4.56	16.03 ± 8.21	43.4 %	25.64 ± 9.89	0.1 %
2	45.00 ± 7.45	33.46 ± 11.01	25.6	32.18 ± 4.89	28.5
3	58.33 ± 8.34	44.62 ± 11.31	23.5	35.38 ± 6.53	39.4
4	58.33 ± 5.89	41.41 ± 11.13	29.0	36.92 ± 8.14	36.7
5	50.00 ± 5.89	38.20 ± 13.72	23.6	33.72 ± 11.37	37.6

$$\text{Inhibitory (\%)} = \frac{\text{Control - Sample}}{\text{Control}}$$

Fig. 1 and 2 show actions of carragheenin to the plantar edema of rat hind legs. Administration of 10 g/kg of OUGON Liquid SE (Concentration powder from OUGON Liquid: 200 mg/kg) gave the result of inhibiting rate: 25.6 % in 2 hours, 23.5 % in 3 hours, 29.0 % in 4 hours i.e. significant difference was observed at the risk of 10 % (t-test) in 2 and 3 hours and, 2 % in 4 hours. Administration of 20 mg/kg of idometacin showed inhibition of 28.5 % in 2 hours, 39.4 % in 3 hours and 36.7 % in 4 hours indicating significant difference in 2 hours at the risk of 2 % (t-test) in 3 and 4 hours at the risk of 1 % (t-test).

E. Discussion

Efficacy of OUGON Liquid SE has been observed with significant difference at a risk of 2 % in inhibition test on rat paw edema. Chinese old literrature on anti-inflammatory action of Ougon reports that Ougon containing baicalein is the medicine for inflammation and Iio et al. mentions that baicalein is one of the most powerful glyoxalse-I inhibitor (the chemical inhibiting histamine release which is a step of most of inflammation). Sekiya et al. reported that the antiallergic action may be revealed by inhibiting production of leukotriens produced by 5-lipoxygenase and the inhibitory action is shown by inhibiting production of lipoxygenase products by indomethacin.

Since OUGON Liquid SE contains baicalein, this anti-inflammatory action was considered due to baicalein.

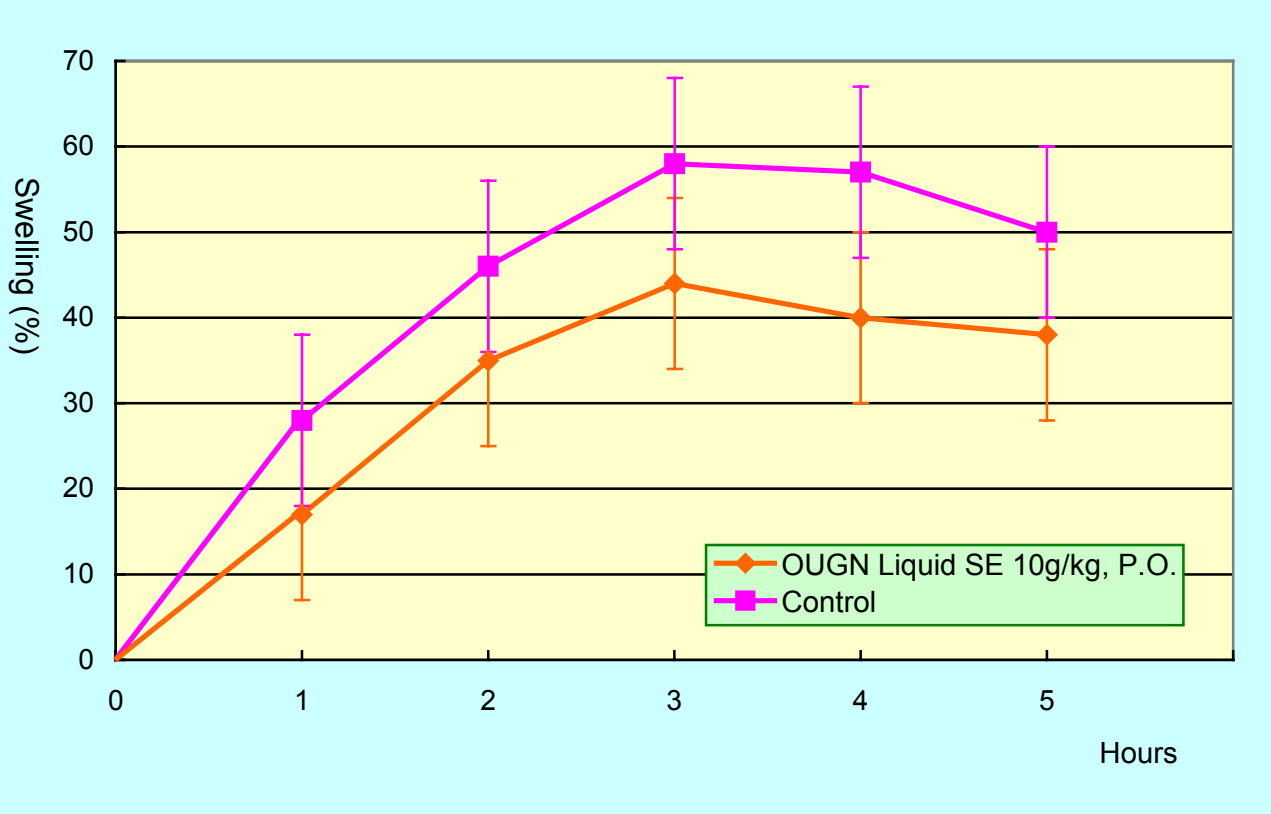


Fig.1 Effect of OUGON Liquid SE on paw edema induced by carrageenin in rats. Each result is the mean value of 5 female rats.

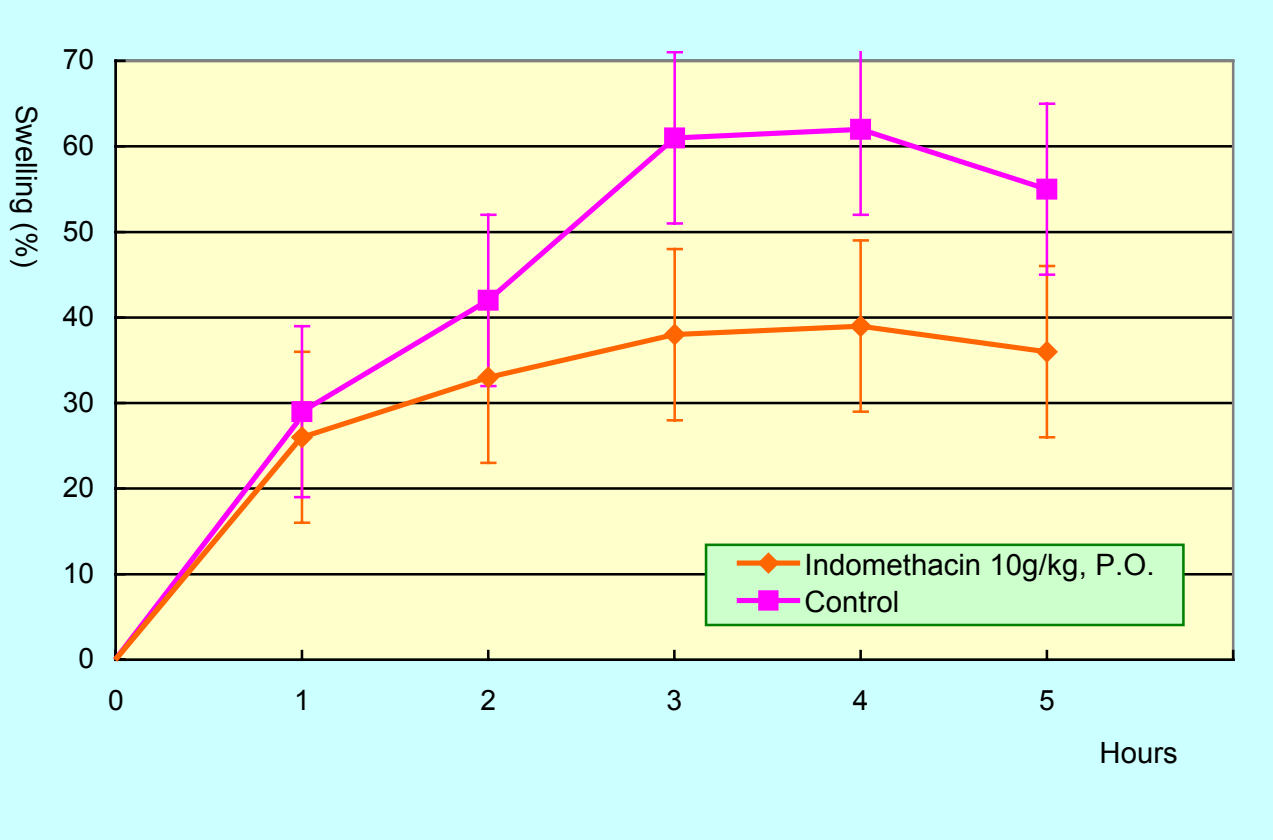


Fig.2 Effect of Indomethacin on paw edema induced by carrageenin in rats. Each result are the mean value of 5 female rats.

4. Suppressive action on the formation of lipid peroxides

Assay of Lipid Peroxides

OUGON Extract Powder was incubated with docosahexaenoic acid for 20 hours, and the mixture of 0.67% thiobarbituric acid (TBA): acetic acid = 1 : 1 was added followed by 95℃ heating for 45 minutes. After cooling in water, n-butanol was added and centrifuged at 1,250 X G for 10 minutes.

The fluorescence of the n-butanol layer was measured using the spectro-fluoro-meter (Hitachi Model NPF4).

Effect of OUGON Extract Powder on TBA reactivity in the medium following 20 hours incubation with docosahexaenoic acid.

Docosahexaenoic acid	Mediator	TBA Reactivity
-	-	0.000
+	-	0.812 ±0.89
+	10^{-6}M	0.74 ± 0.08
+	10^{-5}M	0.69 ± 0.07
+	10^{-4}M	0.63 ± 0.06

Optical density at 535 nm following subtraction of control values for each mediator

Note: Observed value for suppressive action of OUGON Extract Powder on lipid peroxides were relatively small compared with its action to remove active oxygen species, but this will be improved when the preparation form of OUGON Extract Powder is modified.

5.Action to eliminate active oxygen species in the human neutrophil system

Measurement of active oxygen species. (O_2^-, H_2O_2, ·OH and chemiluminascence)

Opsonized zymosan-stimulated neutrophils (polymorphonuclear leukocytes, PMN) were isolated from the venous blood of healthy human subjects according to references [1),2)].

Zymosan used for PMN stimulation was opsonized beforehand with the serum to be tested following references [1),2)].

The PMN was separated by the Ficol-Hypaque gradient density method, and the PMN-containing layer was diluted with the serum to be tested and physiological saline containing 6% dixtran hemolysed by 0.876% ammonium chloride.

The PMN culture solution was prepared by suspending purified PMNs in 5% glucose-containing Krebs Ringer phosphate to be 1 mg/ml. One of active oxygen species, O_2^-, was assayed by means of reduction by cytochrome C using a Beckman spectophotometer. H_2O_2 was assayed by measuring the decrease of scopoletin fluorescence using the spectro-fluorometer (Hitachi Model MPF4). For the assay of ·OH, the amount of ethylene gas (C_2H_4) produced by addition of α-keto-methiol butyric acid was measured by a gas chromatograph (Hitachi).

Chemiluminescence was measured using the liquid scintillation counter (Packard Illions, USA) under tight shield against light.

Effect of OUGON Extract Powder on oxygen radical levels generated by 1mg/ml opsonized zymosan-stimulated neutrophils.

	O_2^- (nmol/10^6P/m*)	H_2O_2 (pmol/2.5X10^6P/m)	·OH (pmol/3X10^6P/m)	Chemiluminescence (10^4CPM/2.5X10^6PMN)
10^{-6}M*$_2$	0.85 ± 0.09	431 ± 74	311 ± 31	22.6 ± 2.9
10^{-5}M*$_2$	0.41 ± 0.04	167 ± 20	175 ± 15	18.7 ± 2.2
10^{-4}M*$_2$	0.62 ± 0.06	0 ± 0	47.1 ± 5.6	11.4 ± 1.0
Control	1.62 ± 0.19	478 ± 43	575 ± 63	25.1 ± 2.7

Control denotes oxygen radical levels generated without addition of the agents.

* P/m=PMN/min

*$_2$ 10^{-6}M : OUGON Extract Powder 0.54 μg

10^{-5}M : OUGON Extract Powder 5.4 μg

10^{-4}M : OUGON Extract Powder 54 μg

1) Niwa Y, Sakane T, Miyachi Y, Kanoh T, and Somiya K: Decrease in generation of oxygen radicals by neutrophils from patients with infections mononucleosis. Role of suppressor T lymphocytes. Blood 64: 994 ～ 999 (1984)

2) Niwa Y, Sakane T, Shingu M, Yanagida I, Komura J, and Miyachi Y: Neutrophilgenerated active oxygens in linear IgA bullous dermatosis. Arch Dermatol 121 : 73 ～ 78 (1985).

6.Anti-Oxidation Effect

Measurement of active oxygen species. (O_2^-, H_2O_2, ·OH and chemiluminascence)

0.1 ml of the hypoxanthine solution (13.5 mg in 50 ml physiological saline), 0.05 ml of 50 mM EDTA solution (50 mM in 2.3 ml physiological saline) and 0.1 ml of 0.72 u xanthine oxidase solution were mixed, and 0.1 ml of this mixture was added to 2 ml of potassium phosphate buffer to produce active oxygen species.

O_2^- was assayed by means of reduction by cytochrome-C using the Beckman spectrophotometer. H_2O_2 was assayed from the decrease of scopoletin fluorescence using the spectro-fluorometer (Hitachi MPF4).

·OH was assayed from the amount of ethylene gas (C_2H_4) produced by addition of α-keto-methiol butyric acid using the Hitachi gas chromatograph.

Effect of OUGON Extract Powder on oxygen radical levels generated in xanthine-xanthine oxidase system.

	O_2^- (nmol/min)	H_2O_2 (pmol/min)	·OH (pmol/min)
10^{-6}M*	8.56 ± 0.94	812 ± 89	1,562 ± 171
10^{-5}M*	4.32 ± 0.46	615 ± 61	776 ± 68
10^{-4}M*	2.02 ± 0.18	0 ± 0	892 ± 107
Control	11.8 ± 1.2	972 ± 116	2,012 ± 221

Control denotes oxygen radical levels generated without addition of the agents.

* 10^{-6}M : OUGON Extract Powder 0.54 μg
10^{-5}M : OUGON Extract Powder 5.4 μg
10^{-4}M : OUGON Extract Powder 54 μg

7. Inhibition of Tyrosinase Activity : Formation of Melanin

Spectrophotometric measurement of the red color due to dopa-chrome formed by reaction between tyrosinase and L-tyrosine. The reaction mixture was incubated for 20 minutes at 37ßC in a water bath, and the optical density (O.D.) was measured at 475 nm.

The percentage inhibition was calculated by comparing with the standard O.D. at 475 nm.

(a) Sample Solution	L-Tyrosine 0.3 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2500 u/ml	:	0.1 ml
	Solution (A)	:	1.0 ml
(b) Blank Solution	Purified Water	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2500 u/ml	:	0.1 ml
	Solution (A)	:	1.0 ml
(c) Standard Solution	L-Tyrosine 0.3 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2500 u/ml	:	0.1 ml
	Solution (B)	:	1.0 ml

Preparation of Solution (A)

OUGON Extract Powder 10 mg, was dissolved in the mixture of Ethanol : 1,3-Butylene glycol : Water = 5 : 3 : 7 to be the final volume of 10 ml.

Preparation of Solution (B)

Ethanol : 1,3-Butyleneglycol : Water = 5 : 3 : 7

OUGON Extract Powder	Inhibition (%)
1.0 mg	67.7 %

8. Reductive De-colorizing Action

Spectrophotometric measurement of the decolation of dopa-chrome formed by reaction between tyrosinase and L-tyrosine.

(a) Sample Solution	L-Tyrosine 0.3 mg/ml	:	1.0 ml
(Forming Dopa-chrome)	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml

The above reaction mixture was incubated for 20 minutes at 37ßC in a water bath, then 1 ml of OUGON Extract Powder solution was added, and the optical density (O.D.) at 475 nm was measured after 1 minute.

(b) Blank Solution	Purified Water	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml

The above reaction mixture was incubated for 20 minutes at 37 ℃ in a water bath, then 1 ml of OUGON Extract Powder solution was added, and the optical density (O.D.) at 475 nm was measured after 1 minute.

(c) Standard Solution	L-Tyrosine 0.33 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml

The above reaction mixture containing the sample (to form Dopa-chrome) was incubated for 20 minutes at 37℃, then 1.0 ml of Ethanol: 1,3-Butylen Glycol: Water = 5 : 3 : 7 was added, and the O.D. at 475 nm was measured after 1 minute.

OUGON Extract Powder	De-coloration
1.0mg	24.2 %

9. Inhibitory Action on the Formation of Melanin polymers

Observation of the formation of melanin polymers by reaction between tyrosinase and L-tyrosine.

(a) Sample Solution	L-Tyrosine 0.3 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml
	Purified Water	:	1.0 ml
	Solution (A)	:	0.01 - 0.30 ml
	Purified Water (*)	:	0.01 - 0.30 ml
	(*) Add the same volume of distilled water as the sample.		

To prepare Solution (A), dissolve 100 mg OUGON Extract Powder in methanol to be the final volume of exactly 50 ml.

After 16 hr incubation at 37℃, the reacted mixture was taken into a 10 ml beaker, and the formation of melanin polymers was observed.

(b) Blank Solution	L-Tyrosine 0.3 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml
	Purified Water	:	1.0 ml
	Methanol	:	0.1 ml

After 16 hr incubation at 37℃, the reacted mixture was taken into a 10 ml beaker, and the formation of melanin polymers was observed.

(c) Standard Solution (Vitamin-C)	L-Tyrosine 0.3 mg/ml	:	1.0 ml
	Mcllvaine Buffer pH 6.5	:	1.0 ml
	Tyrosinase from Mushroom 2480 u/ml	:	0.1 ml
	Purified Water	:	1.0 ml
	Solution (B)	:	0.01 - 0.30 ml
	Purified water (*)	:	0.01 - 0.30 ml

To prepare Solution (B), dissolve 100 mg of L-ascorbic acid in distilled water to be the final volume of 50 ml.

After 16 hr incubation at 37 ℃, the reacted solution was taken into a 10 ml beaker, and the formation of melanin polymers was observed.

(*)Add the same volume of methanol as the vitamin C solution.

Solution (ml)	OUGON Extract Powder (mg)	Melanin Polymers
0.30	0.6	-
0.20	0.4	-
0.10	0.2	-
0.09	0.18	-
0.08	0.16	-
0.07	0.14	-
0.06	0.12	-
0.05	0.10	-
0.04	0.08	-
0.03	0.06	-
0.02	0.04	±
0.01	0.02	+

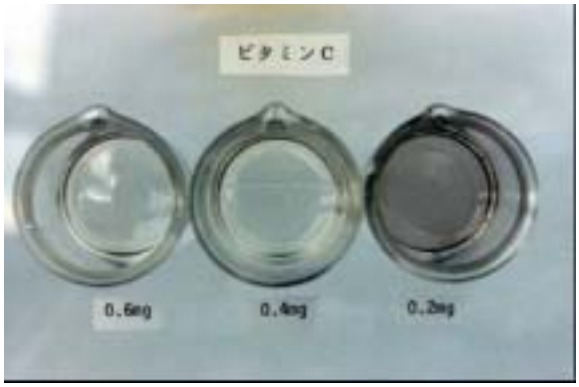
Solution (ml)	Vitamin-C (mg)	Melanin Polymers
0.30	0.6	-
0.20	0.4	-
0.10	0.2	-
0.09	0.18	-
0.08	0.16	-
0.07	0.14	-
0.06	0.12	±
0.05	0.10	+
0.04	0.08	+
0.03	0.06	+
0.02	0.04	+
0.01	0.02	+

(- : Negative　± : Unclear　+ : Positive)

Result (Pictures)

OUGON Extract Powder

(Left:0.6mg　Mid:0.4mg　Right:0.2mg)



Vitamin C

(Left:0.6mg　Mid:0.42mg　Right:0.2mg)

OUGON Extract Powder

(Left:Standard Mid:0.2mg Right:018mg)

Vitamin C

(Left:Standard Mid:0.2mg Right:0.18mg)

OUGON Extract Powder

(Left:0.16mg Mid:0.14mg Right:0.12mg)

Vitamin C

(Left:0.16mg Mid:0.14mg Right:0.12mg)

OUGON Extract Powder

(Left:0.1mg Mid:0.08mg Right:0.06mg)

Vitamin C

(Left:0.2mg Mid:0.1mg Right:0.08mg)

OUGON Extract Powder

(Left:0.06mg Mid:0.04mg Right:0.02mg)

Vitamin C

(Left:0.06mg Mid:0.04mg Right:0.02mg)

Discussion

OUGON Extract Powder was confirmed to have inhibitory actions not only on the activity of tyrosinase but also on the formation of melanin. Moreover, OUGON Extract Powder reductively decolorized tyrosinase-produced dopa-chrome (an intermediate product of melanin for mention). Thus, OUGON Extract Powder is a very effective inhibitor of melanin formation, which not only inhibits the formation of melanin but also is expected to decolorize existing colored melanin.

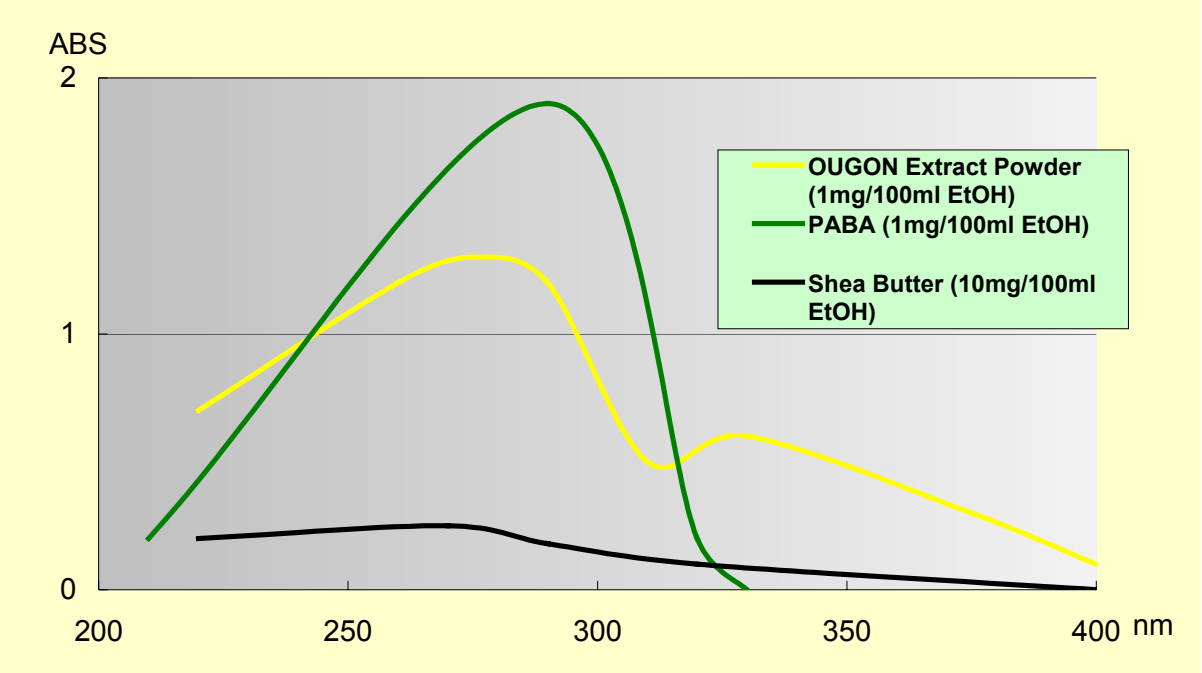
It was also found by the present study that the suppressive action of OUGON Extract Powder on melanin formation was stronger than that of Vitamin-C that is said to have a strong inhibitory action on tyrosinase activity.

10. UV Absorption Effect

1)Measurement for UV absorption

Dissolve 1mg of OUGON Extract Powder in ethanol, to be the final volume 100ml. Then, measure UV absorption using this prepared test solution.

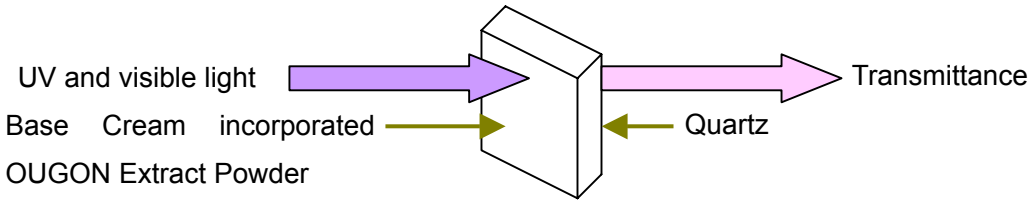
Same as p-aminobenzoic acid (PABA), and Shea Butter of the typical UV absorber, are determined as follows:-

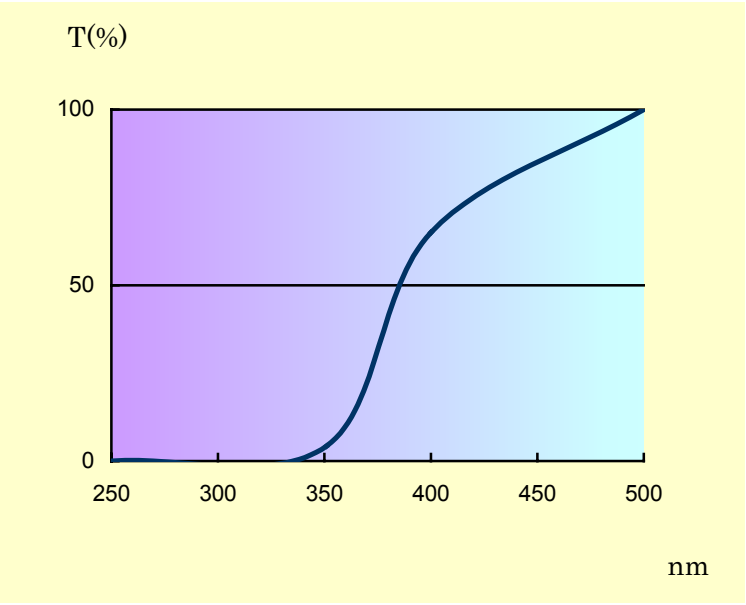


Less than 320nm of UV-B wavelength of OUGON Extract Powder was not as strong a UV-absorber as PABA; graph shows OUGON Extract Powder to be better than PABA in UV-A range. OUGON Extract Powder can absorb Ultraviolet until 400nm.

2) UV absorption on finished product incorporated OUGON Extract Powder

After make base cream that hasn't absorbed UV-A and UV-B, add 1% of OUGON Extract Powder, determined UV and visible light transmittance.





Formulation of Base Cream

Polyvinylpyrrolidone	15g
Polyethylene Glycol	15g
Sodium Alginate	20g
Arabic Gum	qs
Purified Water	450g

UV absorption on finished product containing OUGON Extract Powder shows absorption not only UV-B but also UV-A.

11. Anti-Photoageing Effect

Test Drug

1 % OUGON Extract Powder ethanol water solution

Subjects

Modifications of L.H. Kligman method were employed. Guinea Pigs were subjected before the test day to clipping the of hair off the back in an area of 5 X 5 cm^2. Photo-aging test was divided into 3 groups and processed according to the following.

(A) UV lights + IR-lights irradiation
(B) UV lights + IR-lights irradiation followed by the addition of 0.5ml of test drug
(C) Untreated

Epidermal thickness of guinea pigs irradiated with UV and IR for 8 weeks, and effect of OUGON Extract Powder. (Fig1)

Area ration of guinea pigs elastic fibers irradiated with UV and IR for 8 weeks, and effect of OUGON Extract Powder. (Fig.2)

Results

Fig.3 Epidermal Thickness

	Thickness (μ m)
(A) UV + IR	126.0 ± 18.5
(B) Test Drug and UV + IR	101.7 ± 15.1
(C) Untreated	68.0 ± 10.0

Fig.4 Area Ratio of Elastic Fiber

	Area (%)
(A) UV + IR	17.60 ± 1.50
(B) Test Drug and UV + IR	15.72 ± 1.56
(C) Untreated	14.38 ± 1.18

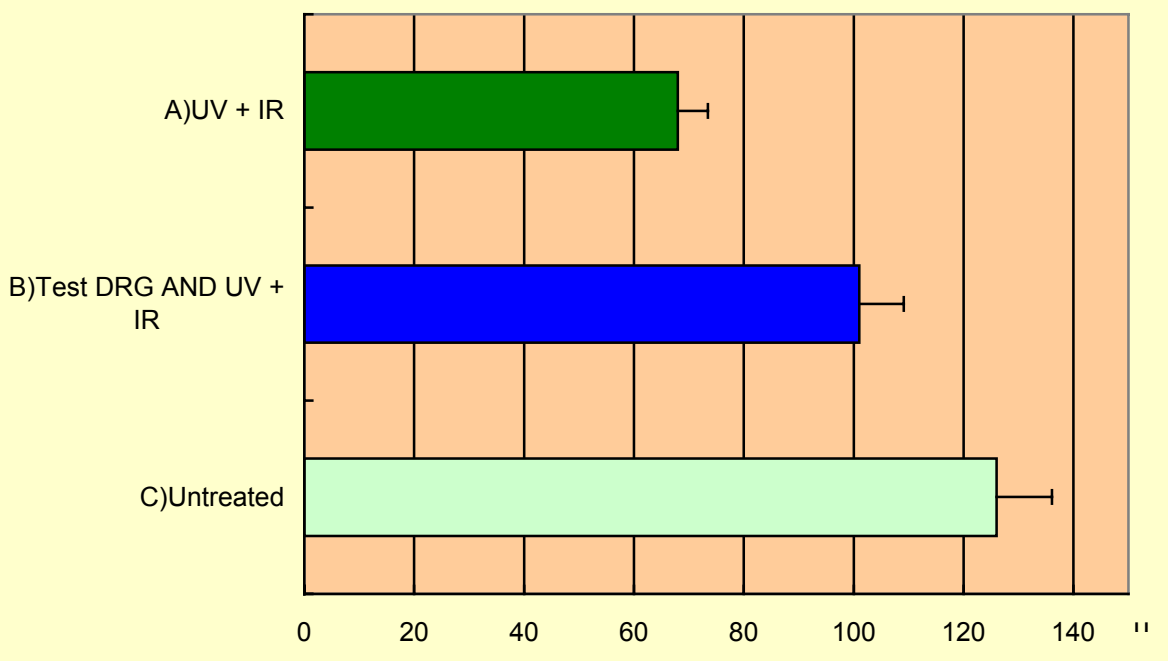


Fig. 3 Epidermal thickness of guinea pigs irradiated with UV and IR for 8 weeks, and effect of OUGON Extract Powder.

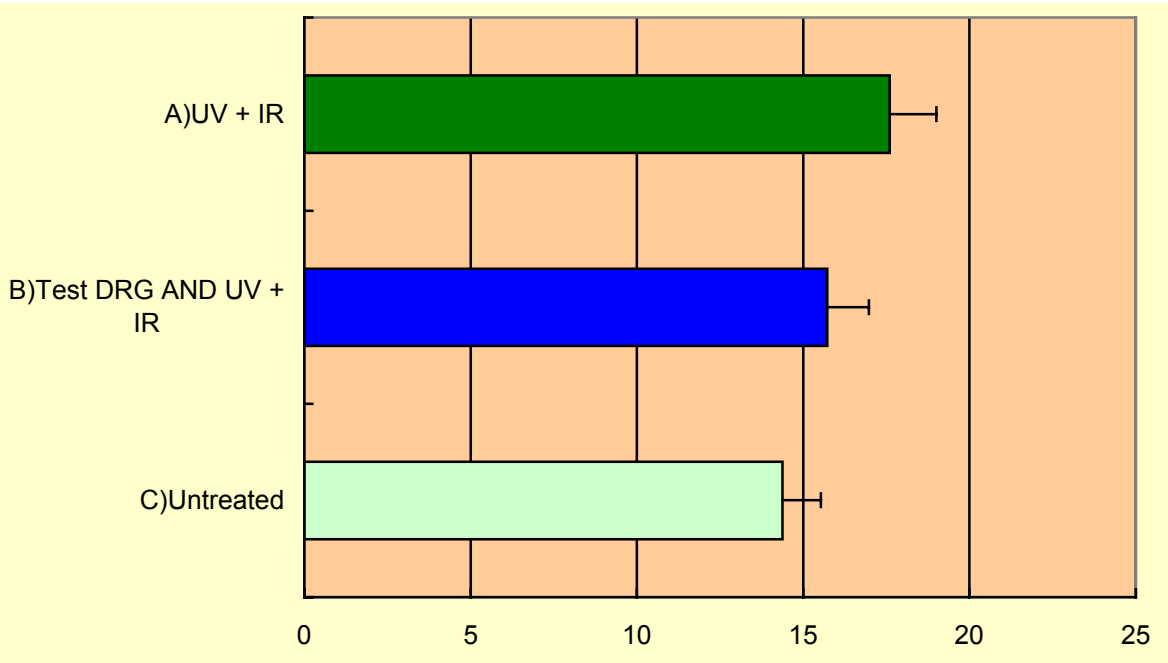
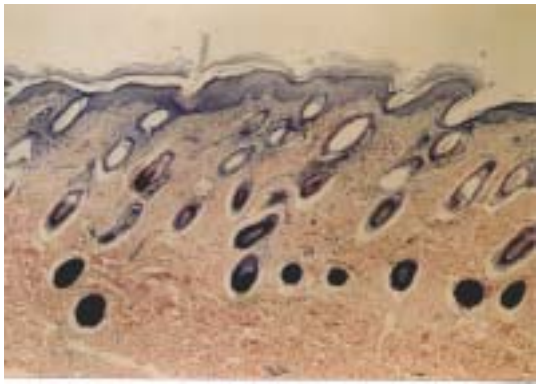
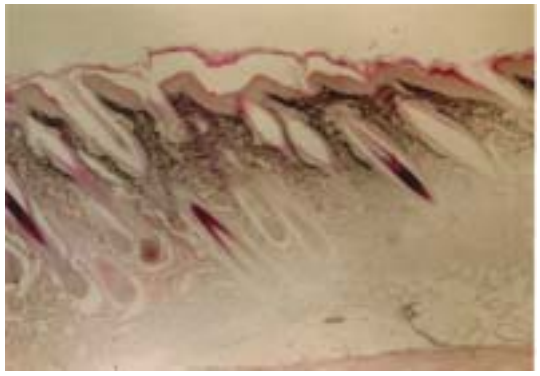


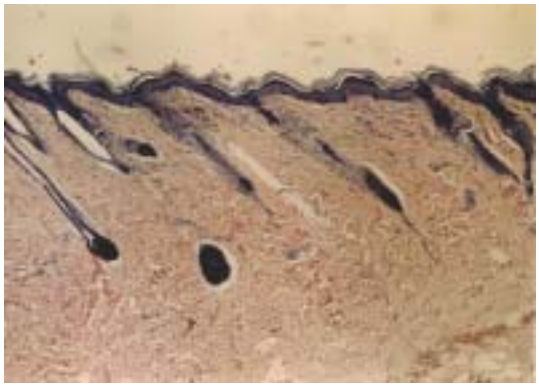
Fig.4 Areal ration of guinea pigs irradiated with UV and IR for 8 weeks, and effect of OUGON Extract Powder.



UV + IR
(Epidermal Thickness)

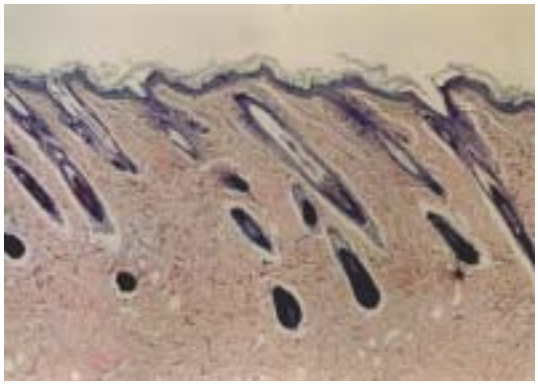


UV + IR
(Areal of Elastic Fiber)

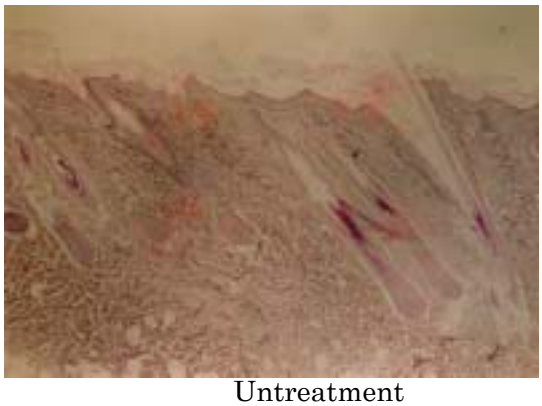


Test Drug and UV + IR
(Epidermal Thickness)

Test Drug and UV + IR
(Areal of Elastic Fiber)



Untraetment
(Epidermal Thickness)



Untreatment
(Areal of Elastic Fiber)

12. Anti-Bacterial Effect

Test Sample

Add 2ml of Glycerin in 3mg of OUGON Extract Powder and dissolve. For control, Glycerin is used.

1) *Escherchia coli*
2) *Bacallus subtilis*
3) *Pseudomonas aeruginosa*
4) *Staphylococcus aureus*

Sterilized medium; add 37g of Bacto Brain Heart Infusion to 1,000ml of purified water and dissolve at 121℃ for 15 minutes by high pressure condition.

Take 10 ml of this medium in test tube, apply bacteria for each test tube, cultivate at 37℃ for 18 hours and as a test solution by 100 times diluted.

Test Method

Add 0.5ml of medium in each 10 test tube (1^{st} to 10^{th}).

Add 0.5 ml in 1^{st} test tube, take 0.5ml from 1^{st} and add 2^{nd}. Twice dilutions make as same process from 2^{nd} to 10^{th} and renounce 0.5ml from 10^{th}.

Dilution of 10 test tubes are as follows: - (1 : 1, 1 : 2,1 : 4, 1 : 8, 1 : 16, 1 : 32, 1 : 64, 1 : 128, 1 : 256 and 1 : 512).

Add 0.5 ml of test solution and control to each 10 test tube. Cultivate them at 37℃ for 18 hours and measure maximum dilution; which show anti-growth bacteria.

Anti-bacterial effect is observed for *Staphylococcus aureus* and *Escherichia coli* on 0.375 - 0.75 ml (0.0375 - 0.075%) and weak anti-bacterial effect for *Bacillus subtilis*.

Result

Bacterial \ Dilution		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Escherichia coli	Sample	-	-	+	+	+	+	+	+	+	+
	Control	-	+	+	+	+	+	+	+	+	+
Bacillus subtilis	Sample	-	±	-	+	+	+	+	+	+	+
	Control	-	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	Sample	-	-	+	+	+	+	+	+	+	+
	Control	-	-	+	+	+	+	+	+	+	+
Staphylococcus aureus	Sample	-	-	-	-	-	+	+	+	+	+
	Control	-	+	+	+	+	+	+	+	+	+

(-:Not growth, ±:Unclear, +:Growth)

Discussion

Ougon is know anti-bacterial effect in Chinese folk documents and have a little wide anti-bacterial effect range.

According to this test and Chinese documents, Ougon has anti-bacterial effect for some of bacteria.

13. Product Specifications

	OUGON Liquid E	OUGON Liquid B	OUGON Liquid SE	OUGON Ex.Powder
Solution Composition	50 % Ethanol	50 % 1,3-BG.	70 % Ethanol	Powder
Amino Acid	Positive	Positive	---	---
Proline	Positive	Positive	---	---
Saccharides	Positive	Positive	Positive	---
Baicaline	Positive	Positive	---	---
Woogonin / Baicalein	---	---	Positive	Positive
Flavonoids	---	---	Positive	Positive
Heavy Metals	20 ppm max.	20 ppm max.	20 ppm max.	20 ppm max.
Arsenic	2 ppm max.	2 ppm max.	2 ppm max.	2 ppm max.
Specific Gravity (d_{20}^{20})	0.920 - 0.940	1.010 - 1.050	0.87 - 0.91	---
Evaporation Residue	1.00 - 1.80	0.50 - 1.30	1.0 - 2.0	---
Loss on drying	---	---	---	5.0 % max.
Alcohol Number	5.0 - 5.6	---	6.9 - 7.9	---
Baicalin	0.10 - 0.20	0.15 - 0.25	---	---
Baicalein	---	---	0.25 - 0.35 %	40 - 70 %
Preservaties	None	None	None	---