ORYZA OIL & FAT CHEMICAL CO., LTD.

PERILLA SEED EXTRACT

Antibacterial Oral Preparation

- PERILLA SEED EXTRACT-PO
  (Powder, Food Grade)
- PERILLA SEED EXTRACT-LO
  (Liquid, Food Grade)
- PERILLA SEED EXTRACT-POC
  (Powder, Cosmetic Grade)
- PERILLA SEED EXTRACT-LOGC
  (Liquid, Cosmetic Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.
Ver. 4.0 YF

PERILLA SEED EXTRACT
Antibacterial Oral Preparation

1. Introduction

Perilla (***Perilla frutescens***), which is a kind of Japanese herb, has been grown widely in Japan, China and Southeast Asia area and used as oil crop and/or folk medicine. It could be said that Perilla is one of the most familiar to almost Japanese.

We, Oryza Oil & Fat Chemical Co., Ltd, focused on Perilla seed and have researched its functions. Our research revealed that polyphenols contained in Perilla seed can inhibit 5-lipoxygenase activity and thus have anti-allergic effect and anti-inflammatory effect.

We also recently demonstrated that Perilla seed has antibacterial activity against various pathogenic microorganisms and started to produce PERILLA SEED EXTRACT for antibacterial too.


Perilla

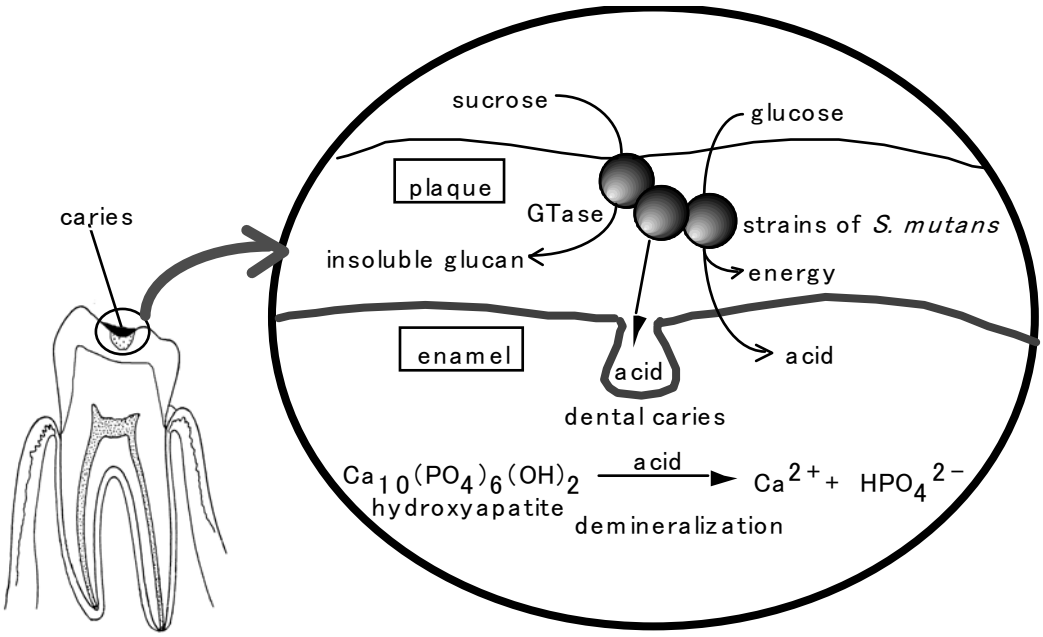

Perilla seeds

2. Pathophysiology of Dental Caries

S. mutans is normally present in the human oral cavity. It produces insoluble glucan from sugar and adheres to the teeth. It is said that druse of microorganisms are formed and proliferate when other microorganisms adheres around there, resulting in plaques formation. *S. mutans* in plaques produces acids from glucose together with energy, so that it keeps in acidity around there. And it is thought that hydroxyapatite, one of ingredients constituting enamel, is decalcified under pH 5.5, and thus dental caries occurs.

PERILLA SEED EXTRACT has been shown to inhibit the growth of cariogenic microorganisms and can be expected to prevent dental caries.

Pathophysiology of Dental Caries

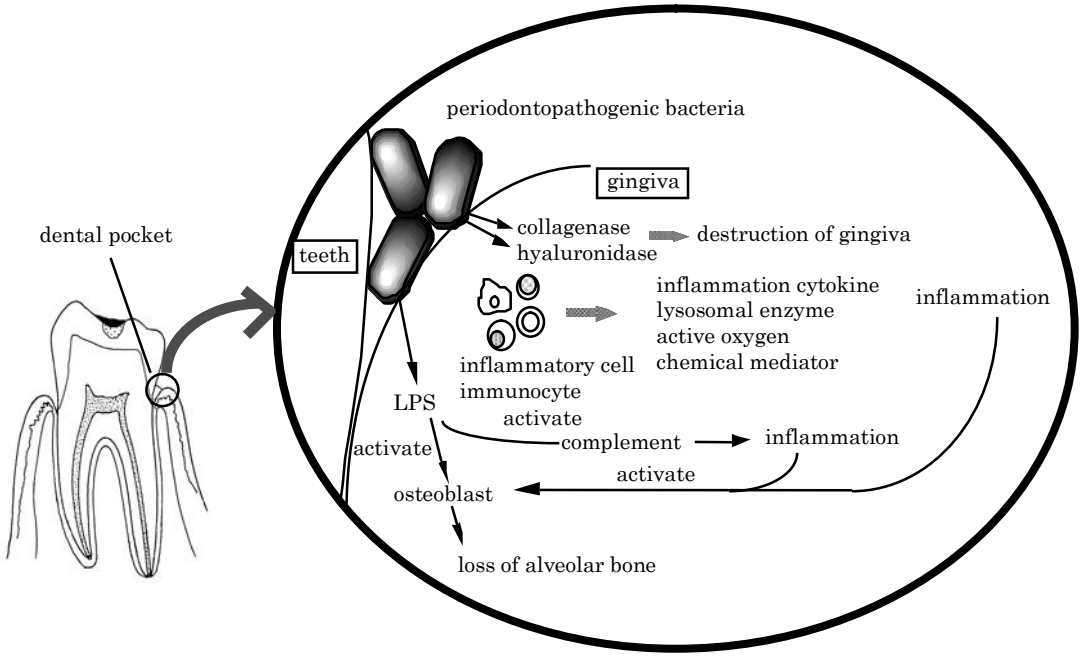


3. Pathophysiology of Periodontal Diseases

Periodontal (gum) diseases including gingivitis and periodontitis are serious diseases if left untreated. It is said that periodontopathogenic bacteria proliferate in periodontal pocket and occur inflammation. The inflammation activates osteoclast, one of cells can destroy bone, and thus dissolve alveolar bone supporting the teeth, finally resulting in loosing the teeth in many cases. Professional treatment and good oral hygiene is normally recommended.

Polyphenols containing in PERILLA SEED EXTRACT have been shown to inhibit the proliferation of periodontopathic microorganisms and reduce inflammation on gums , and it is expected that PERILLA SEED EXTRACT can improve periodontal diseases.

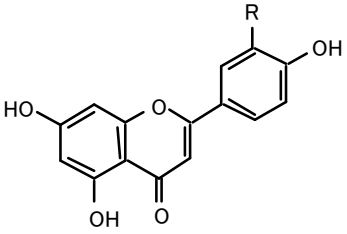
Pathophysiology of Periodontal Diseases

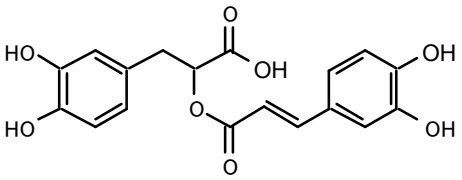


4. Active Components of PERILLA SEED EXTRACT

PERILLA SEED EXTRACT is rich in polyphenols such as luteolin, chrysoeriol and apigenin as aglycons. Luteolin and so on are main polyphenols and main active ingredients in PERILLA SEED EXTRACT to show anti-dental caries and anti-periodontal disease effect.

Structures of Polyphenols from Perilla Seed





R =OH Luteolin

OCH_3 Chrysoeriol

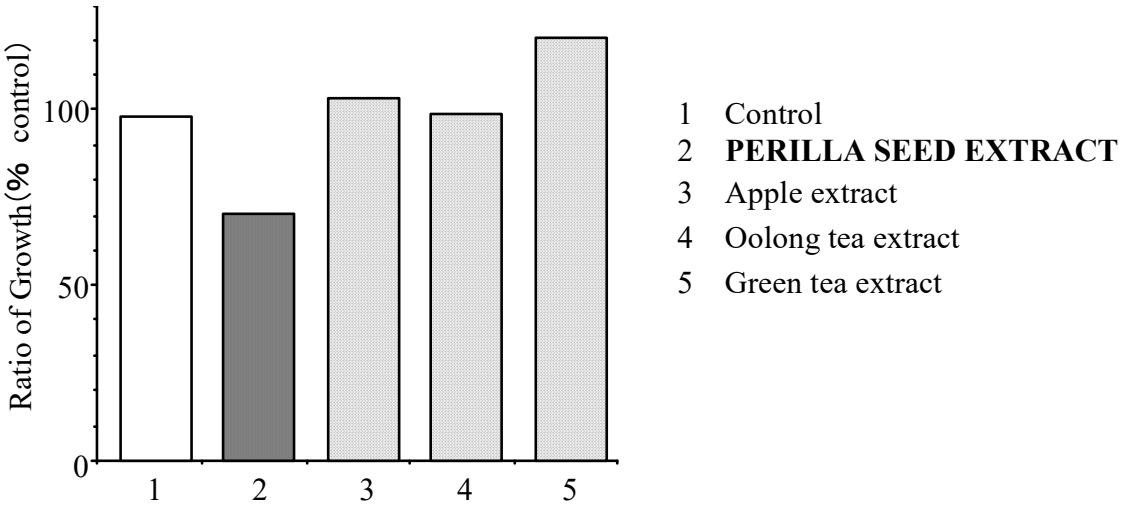
H Apigenin

Rosemarinic acid

5. Effect of PERILLA SEED EXTRACT on Dental Caries

5-1 Effect of PERILLA SEED EXTRACT on *S. mutans*

The effect of various plant extracts on ***S. mutans GS5*** growth was compared and examined. We recognized that PERILLA SEED EXTRACT showed stronger inhibitory effect on growth of *S. mutans* GS5 than apple extract, oolong tea extract and green tea extract.



Effect of Various Extracts on Growth of *S. mutans* GS5

<Method>

Each sample was added at concentration 200 μg/ml to 5 ml BHI broth inoculated on ***S. mutans*** GS5 10^4 cells and incubated at 37 ℃ for 9 hours. OD600 was measured and ration of growth was calculated.

5-2 Effect of PERILLA SEED EXTRACT on Oral Streptococci

PERILLA SEED EXTRACT showed strong antimicrobial activity against cariogenic bacteria like *S. mutans* and *S. sobrinus*. We also demonstrated that luteolin which is one of polyphenols mainly containing in PERILLA SEED EXTRACT showed the most strong antimicrobial activity against cariogenic bacteria among samples we tested. We found that the antimicrobial activity of luteolin is stronger than rosemarinic acid known to contain in Perilla leaf extract.

MIC of Perilla Seed Polyphenols against Oral Streptococci

		MIC (μg/ml)				
	Strain	ethanol ext.	ethylacetate ext.	luteolin	rosemarinic acid	EGCg
S. mutans	GS-5	1600	100	100	1600	200
	OMZ-175	1600	100	100	＞1600	400
	ATCC 25175	1600	200	100	＞1600	800
	ATCC 27352	400	50	50	1600	400
S. sobrinus	OMZ-176	1600	100	100	1600	400
S. sanguis	ATCC 10556	＞1600	200	400	1600	200
	ATCC 10557	＞1600	800	100	＞1600	1600
	AU 1023	＞1600	800	400	＞1600	1600
S. salivarius	ATCC 7073	＞1600	400	800	＞1600	200
	AU 2163	＞1600	100	200	＞1600	800
S. mitior	ATCC 9811	＞1600	200	200	＞1600	800
S. oralis	ATCC 35037	1600	400	200	1600	400
	AU 1903	＞1600	400	200	＞1600	800

6. Effect of PERILLA SEED EXTRACT on Periodontal Diseases

6-1 Effect on Periodontopathogenic Bacteria

P. gingivalis is known to isolate from 74 % people with periodontal diseases and PERILLA SEED EXTRACT showed to inhibit proliferation of *P. gingivalis*. We also found that PERILLA SEED EXTRACT can inhibit proliferation of *F. nucleatum* which is thought to be one of periodontopathogenic bacteria and the inhibitory effect of PERILLA SEED EXTRACT is much stronger than perilla leaf extract. Luteolin also showed strong antimicrobial activity against periodontopathogenic bacteria, and thus we suggested that luteolin containing in PERILLA SEED EXTRACT is one of the most

important ingredient for the antimicrobial activity of PERILLA SEED EXTRACT.
And we recently found that PERILLA SEED EXTRACT can inhibit growth of *P. gulae* and *P. salivosa* known to be in dog mouth and cause dog periodontal diseases.

MIC of PERILLA SEED EXTRACT
against periodontopathic microorganisms

	strain	MIC (μg/ml)					
		Perilla seed ethanol ext.	ethylacetate ext.	luteolin	rosemarinic acid	EGCg	Perilla leaf ethanol ext.
P. gingivalis	BH 18/10	800	100	25	800	50	
	RB 24M-2	1600	100	25	800	100	
	OMZ 314	800	50	12.5	800	200	
	W 50	1600	50	25	800	200	
	6/26	1600	25	50	1600	100	
	381	1600	25	50	800	100	
F. nucleatum	ATCC25586	800		400			6400
P. gulae	ATCC51700	1250					
P. salivosa	ATCC49407	10000					

6-2 Superoxide Radical Scavenging Activity

PERILLA SEED EXTRACT and luteolin, one of the main ingredients in PERILLA SEED EXTRACT showed high superoxide scavenging activity illustrated as below:

We suggested that the anti-inflammatory effect of PERILLA SEED EXTRACT is mainly due to its strong radical scavenging activity of luteolin.

Superoxide Scavenging Action of PERILLA SEED EXTRACT

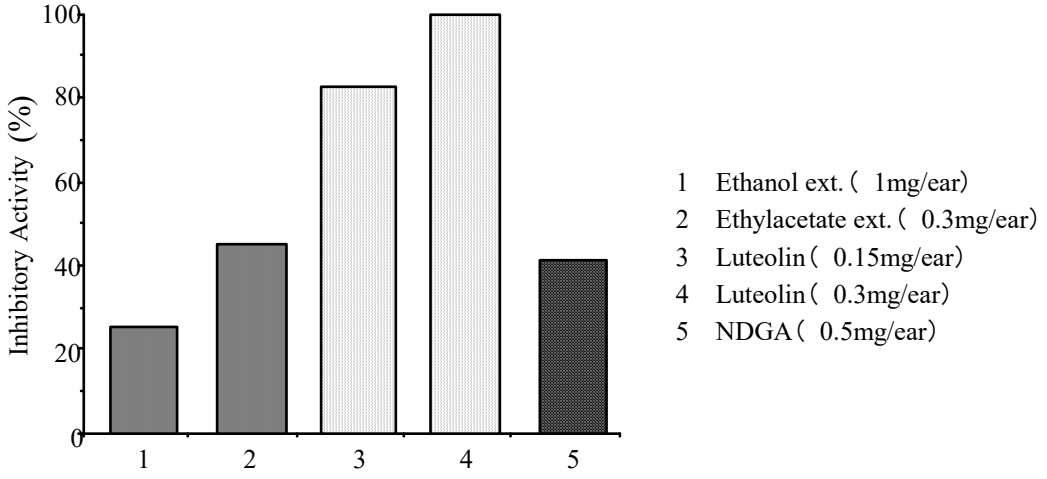
	(unit/g)
PERILLA SEED EXTRACT	46,000*
Luteolin	80,420**

*NBT method **ESR method

6-3 Anti-inflammatory Effect

TPA, a type of chemotactic factor and reactive oxygen species are commonly released from leukocyte in response to an allergic reaction. PERILLA SEED EXTRACT has been shown to reduce TPA induced inflammation in mouse skin.

Luteolin is said to be one of the highest 5- and 12- lipoxigenase inhibitor among natural ingredients and very strong radical scavenger, and thus we suggested that the anti-inflammatory effect of PERILLA SEED EXTRACT is due to the inhibitory activities of 5- and 12- lipoxigenase needing to produce leukotrienes and the activity to scavenge radicals produced by leukotrienes.



Effect of PERILLA SEED EXTRACT and Luteolin on TPA- induced Inflammation in Mouse

7. Effect on Deodorization

PERILLA SEED EXTRACT has been shown to have deodorizing effect against acetaldehyde, ammonia and methylmercaptan, which are commonly found in tobacco, alcohol, sweat and bad breath.

Ingredients	Preparation
acetaldehyde	Tobacco, Alcohol
ammonia	Tobacco, Alcohol and Sweat
methylmercaptan	Bad breath

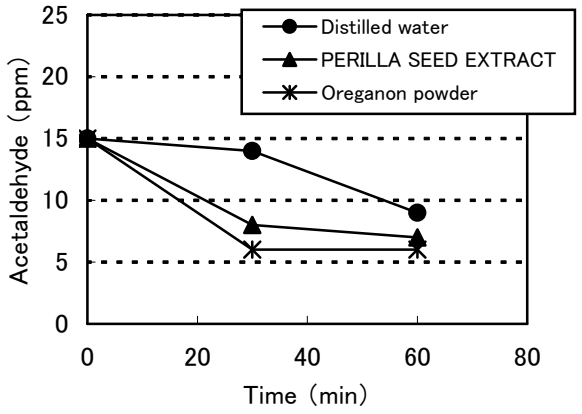
PERILLA SEED EXTRACT demonstrated that deodorizing effect similar to that of Oreganon powder against acetaldehyde, ammonia and methylmercaptan.

Thus, we found that PERILLA SEED EXTRACT is an effective deodorizing agent against tobocco, alcohol, sweat and bad breath.

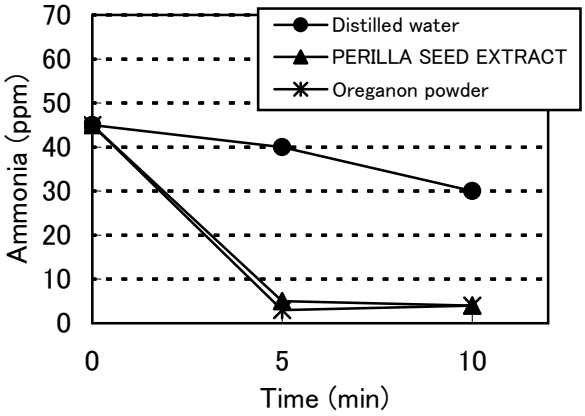
<Methods>

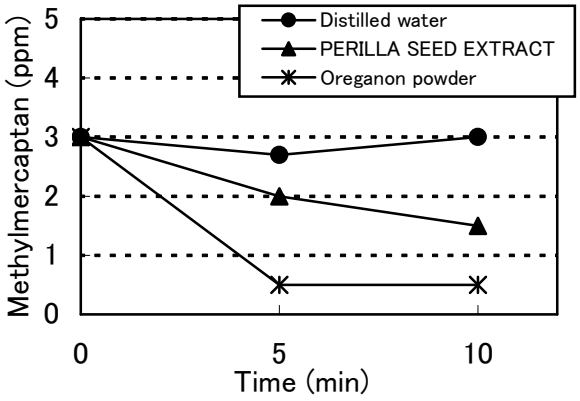
After deodorization sample is dissolved in distilled water, smelly substance is added. The concentration of acetaldehyde, ammonia and methylmercaptan in headspace is measured with Gas Detector Tubes.

Effect against acetaldehyde



Effect against ammonia





Effect against methylmercaptan

8. Application of PERILLA SEED EXTRACT

The antimicrobial activity of PERILLA SEED EXTRACT on ***S.mutans*** effectively prevent the formation of dental caries. Meanwhile, its potent radical scavenging activities and anti-inflammatory effects prevent against periodontitis and promotes good oral hygiene.

PERILLA SEED EXTRACT is suitable to be incorporated into food and beverage preparations such as chewing gums, candies, juice and dental care products (e.g. toothpaste, mouthwash etc.) for the maintenance of dental and periodontal health. agent.

9. Effect of PERILLA SEED EXTRACT in Oral Care Preparations

antimicrobial activity

The effect of oral care preparations containing PERILLA SEED EXTRACT was examined. Each preparation containing various concentrations of PERILLA SEED EXTRACT was used (to simulate actual dilution in mouth). The antimicrobial activity of each preparation on *S.mutans* was examined. And thus we found that antimicrobial activities of toothpastes with PERILLA SEED EXTRACT were higher than that of toothpaste only.

<Method>
Each sample of toothpastes containing distilled water, 0.1% PERILLA SEED EXTRACT and 0.2% PERILLA SEED EXTRACT respectively was treated with *S.mutans*. After incubation at RT for 24 hours, aliquots of samples were streaked on nutrient agar and further incubated for 48 hours. Growth of bacteria was measured.

Effect of Toothpastes product containing PERILLA SEED EXTRACT against *S. mutans*

Sample: Toothpaste A (Base No PERILLA SEED EXTRACT)

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTROL	PRODUCT	CONTROL	PRODUCT
Initial	8.4×10^4	---	---	---	---	---
05 min	7.1×10^4	6.6×10^4	15.48	21.43	0.07	0.10
01 hour	6.8×10^4	5.9×10^4	19.05	29.76	0.09	0.15
48 hours	1.3×10^4	8.0×10^1	84.52	99.90	0.81	3.02

Sample: Toothpaste B (0.10% PERILLA SEED EXTRACT)

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTRO	PRODUCT	CONTROL	PRODUCT
Initial	8.4×10^4	---	---	---	---	---
05 min	7.1×10^4	5.6×10^4	15.48	33.33	0.07	0.18
01 hour	6.8×10^4	3.7×10^4	19.05	55.95	0.09	0.36
48 hours	1.3×10^4	3.0×10^1	84.52	99.96	0.81	3.45

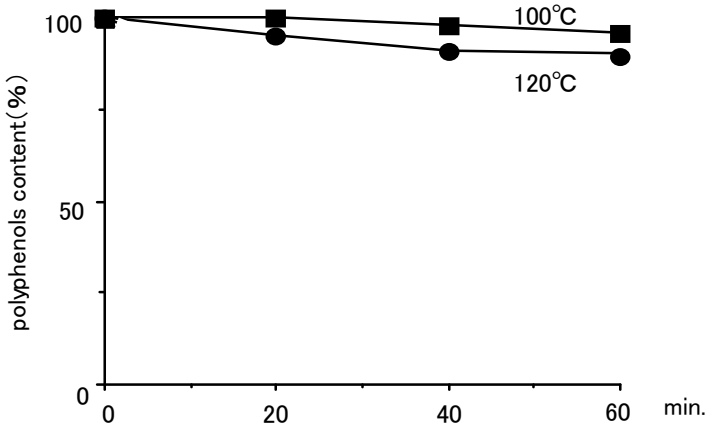
Sample: Toothpaste C (0.20% PERILLA SEED EXTRACT)

EXPOSURE TIME	CONCENTRATION OF ORGANISM (CFU/mL)		% REDUCTION		LOG REDUCTION	
	CONTROL	PRODUCT	CONTRO	PRODUCT	CONTROL	PRODUCT
Initial	8.4×10^4	---	---	---	---	---
05 min	7.1×10^4	4.8×10^4	15.48	42.86	0.07	0.24
01 hour	6.8×10^4	4.1×10^2	19.05	99.50	0.09	2.31
48 hours	1.3×10^4	<10	84.52	99.99	0.81	3.92

10. Stability of PERILLA SEED EXTRACT

10-1 Heat Resistance

PERILLA SEED EXTRACT remain stable at high temperature, 100 ℃ and 120 ℃.

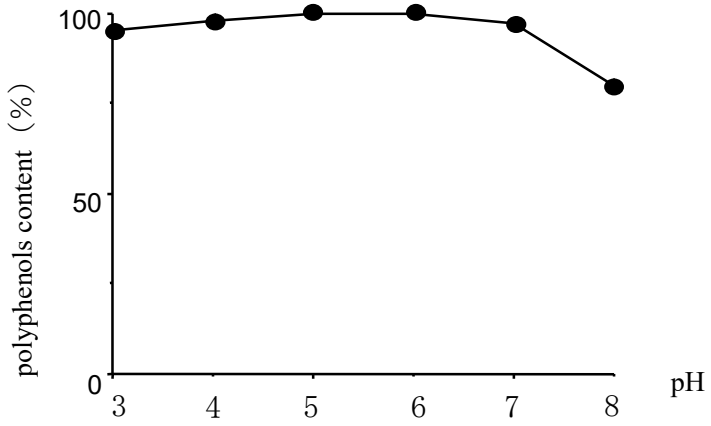


X-axis: % of initial polyphenols

Heat-Resistance of PERILLA SEED EXTRACT

10-2 pH Stability

Polyphenols containing in PERILLA SEED EXTRACT are highly stable at pH 3-7.



X-axis: % of PERILLA SEED EXTRACT polyphenols
in 0.05% solution (30% aqueous ethanol)

Effect of pH on The Polyphenols Content

11. Daily Dosage of PERILLA SEED EXTRACT

PERILLA SEED EXTRACT-PO (powder)

Recommended dosage : 80-160 mg / day

e.g., Toothpaste : 3x 25 mg-50 mg daily, hence 2.4 - 4.8 g per tube

Candy/Chewing Gum : 13-27 mg/piece, maximum 6 pcs /day

PERILLA SEED EXTRACT-LO (liquid)

Recommended dosage : 120 mg-240 mg/day

e.g., Toothpaste : 3x 40-80 mg daily, hence 3.6 - 7.2 g per tube

Candy or Chewing Gum : 20-40 mg/piece, maximum 6 pcs/day

12. Nutrition Facts of PERILLA SEED EXTRACT

PERILLA SEED EXTRACT-PO

Items Analyzed	Result
Water	3.1 g/100g
Protein*1	0.9 g/100g
Fat	0.2 g/100g
Ash	0.8 g/100g
Available carbohydrate*2	95.0 g/100g
Energy*3	385 kcal/100g
Dietary Fiber	0 g/100g
Sodium	7.1 mg/100g

PERILLA SEED EXTRACT-LO

Items Analyzed	Result
Water	79.0 g/100g
Protein*1	0.6 g/100g
Fat	0.1 g/100g
Ash	0.5 g/100g
Available carbohydrate*2	19.8 g/100g
Energy*3	83 kcal/100g
Dietary Fiber	0 g/100g
Sodium	4.7 mg/100g

*1 N×6.25

*2 100-(Moisture + Protein + Fat + Ash + Dietary fiber)

*3 Factors for calculating the energy value:
Protein - 4, Fat - 9, Available carbohydrate -4
Tested by : Japan Food Research Center Foundation
Research result issue number : 397060549-001

13. Safety of PERILLA SEED EXTRACT

13-1 Residual Agricultural Chemicals

Assayed Items	Results	Detection Limits	Assay Method
BHC	Not Detected	0.02 ppm	Gas Chromatography
DDT	Not Detected	0.02 ppm	Gas Chromatography
Aldrin	Not Detected	0.01 ppm	Gas Chromatography
Dieldrin	Not Detected	0.01 ppm	Gas Chromatography
Endrin	Not Detected	0.01 ppm	Gas Chromatography
Diazinon	Not Detected	0.05 ppm	Gas Chromatography
Parathion	Not Detected	0.05 ppm	Gas Chromatography
Malathon	Not Detected	0.05 ppm	Gas Chromatography

Tested by: Japan Food Research Center Foundation

13-2 Acute Toxicity and Safety

No toxic effects observed at 2000 mg/kg body weight for 14 days. Thus, it is deduced that LD_{50} (in mouse) is more than 2000 mg/kg body weight.

No toxic effects observed at high doses of 7.0 g/day in human for 14 days.

14. Practical Applications of PERILA SEED EXTRACT

Applications	Examples
Confectionery	Candy, Gum, Gummi, Cookie, Wafer, Pudding, Jelly, Yogurt, etc.
Drinks	Functional drink, Nutritional drink, etc.
Health foods	Tablet(Chewable type), etc.
Oral Care Products	Toothpaste, Mouthwashes, etc

15. Packing

PERILLA SEED EXTRACT-PO, -POC

5 kg interior packaging : a double layered plastic bag, can
exterior packaging : cardboard box

PERILLA SEED EXTRACT-LO, -LOGC (liquid)

5 kg interior packaging : cubic polyethylene container
exterior packaging : cardboard box

16. Storing Method

Store in cool, dry place. Avoid humidity.

17. Expression of PERILLA SEED EXTRACT

PERILLA SEED EXTRACT-PO: Perilla seed extract and treharose

PERILLA SEED EXTRACT-LO: Ethanol and perilla seed extract

*Please refer to your nation’s standard.

PRODUCT STANDARD

PRODUCT NAME

PERILLA SEED EXTRACT-PO

(FOOD)

This product is extracted from the seeds of perilla (*perilla frutescens* var. *japonica*, *p.frutescens* var. *frutescens* or *perilla frutescens* var. *crispa*) with aqueous ethanol. It guarantees a minimum of 3.0 % polyphenols.

Appearance	Yellowish powder with slightly unique smell
Certification Test	To 1.0 g of this product, add 8.5 ml of 90 % diethlene glycol and 0.5 ml of 1N NaOH. Then it changes yellow. (DAVIS method)
Polyphenols	Min. 3.0 % (Folin-Denis method)
Loss on Drying	Max. 5.0 % (Analysis for Hygienic Chemists,1g, 105℃, 2h)

Purity Test

(1) Heavy Metals (as Pb)	Max. 30 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)

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

Ingredients	Contents
Trehalose	70 %
Perilla Seed Extract	30 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

PERILLA SEED EXTRACT-LO

(FOOD)

This product is extracted from the seeds of perilla (*perilla frutescens* var. *japonica*, *p.frutescens* var. *frutescens* or *perilla frutescens* var. *crispa*) with aqueous ethanol. It guarantees a minimum of 2.0 % polyphenols.

Appearance	It is dark brown liquid with slightly unique smell.	
Certification Test (Flavonoids)	To 1.0 g of this product, add 8.5 ml of 90 % diethlene glycol and 0.5 ml of 1N NaOH. Then it changes yellow.	(DAVIS method)
Content of Polyphenols	Min. 2.0 %	(Folin-Denis method)
Residue on Evaporation	18.0～22.0 %	(Analysis for Hygienic Chemists)

Purity Test

(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

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

Ingredients	Contents
Ethanol	50 %
Purified water	30 %
Perilla Seed Extract	20 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

PERILLA SEED EXTRACT-POC

(COSMETIC)

This product is extracted from the seeds of perilla (Perilla frutescens var. frutescens) with aqueous ethanol. It contains not less than 3.0 % of Polyphenols.

Appearance	Yellowish powder with slightly characteristic odor
Identification Flavonoid	To 1.0 g of this product, add 8.5 mL of 90 % diethylene glycol and 0.5 mL of 1 N NaOH. Then it changes yellow. (DAVIS method)
Polyphenols	Min. 3.0 % (Folin-Denis method)
Loss on Drying	Max. 5.0 % (Analysis for Hygienic Chemists,1g, 105℃, 2h)

Purity Test

(1) Heavy Metals (as Pb)	Max. 30 ppm (Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

Standard Plate Counts	Max. 1×10^2 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

Ingredients	Contents
Trehalose	70 %
Perilla Ocymoide Seed Extract	30 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

PERILLA SEED EXTRACT-LOGC

(COSMETIC))

This product is a mixture of an extract obtained from the seeds of perilla (Perilla frutescens var. frutescens)with ethanol solution, glycerine and water. It contains sodium benzoate and potassium sorbate as preservative.

It comprises not less than 2.0% of polyphenol in the product.

Appearance	Dark brown liquid with characteristic odor.	
Certification Test (Flavonoids)	To 1.0 g of this product, add 8.5 ml of 90 % diethlene glycol and 0.5 ml of 1N NaOH. Then it changes yellow.	(DAVIS method)
Content of Polyphenols	Min. 2.0 %	(Folin-Denis method)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 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

Ingredients	Contents
Glycerin	49.2 %
Water	30 %
Perilla Ocymoides Seed Extract	20 %
Sodium Benzoate	0.4 %
Potassium Sorbat	0.4 %
Total	100 %

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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ORYZA OIL & FAT CHEMICAL CO., LTD.