TEST REPORT

APPLICANT	:	P.A. & S.C. STEENS LIMITED
ADDRESS	:	298 Manaia Road, RD5, Masterton 5885 ,New Zealand
SAMPLE NAME	:	STEENS UMF20+ RAW MANUKA HONEY
SAMPLE STATES	:	Liquid
RECEIVED DATE	:	10/13/2023
PRODUCTION DATE OR BATCH NUMBER	:	/
EXPIRY DATE	:	/
SPECIFICATION	:	225g/bottle
SAMPLE QUANTITY	:	1 bottle
TEST RESULT	:	Please see the next page
CONCLUSION	:	Please see the next page

The following test item(s) was/were performed on submitted sample(s) and/or component(s) confirmed by applicant

Test Item	Requirement	Test Conclusion
In vitro antioxidant benefits	Laboratory methods (CALT/TM/SOP274-01 Antioxidant Capacity-Hydroxyl Radical Method)	It has scavenging ability, that is, under the conditions of this test, it has antioxidant capacity.
In vitro anti-aging efficacy assessment	Laboratory methods (CALT/TM/SOP282-01 Standard operating procedures for the in vitro hyaluronidase inhibition assay)	It has inhibitory effect, that is, under the experimental conditions, the sample has an anti-aging capacity.

********** FOR FURTHER DETAILS, PLEASE REFER TO THE FOLLOWING PAGE(S) **********

Signed for and on behalf of
Eurofins Cosmetic Testing Service (Shanghai) Co., Ltd.

Approver:

Shirley Meng

TEST REPORT

Test item 1 In vitro antioxidant benefits

Appendix:

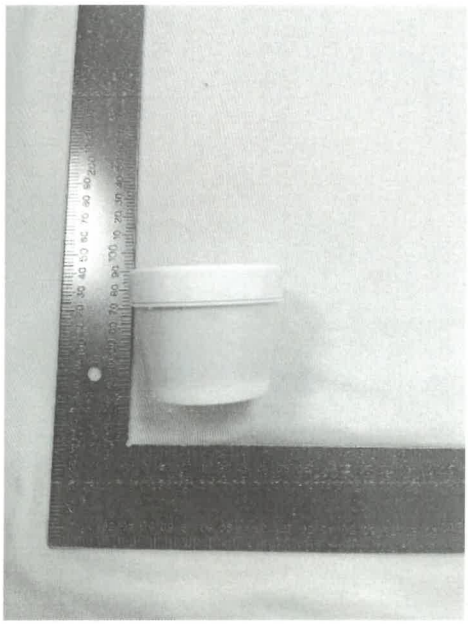
1. Basic sample information

Sample Name:	STEENS UMF20+ RAW MANUKA HONEY	L/N:	/
Chemical Name:	/	Physical state:	Viscous liquid
CAS No.:	/	Colour:	Dark yellow
INCI name:	/	Purity:	/
Product name:	/	Storage Conditions:	Room temperature
Relative molecular weight:	/	Stability:	Stable under storage conditions
Molecular formula:	/	Production Date:	/
Manufacture:	P. A. & S. C. STEENS LIMITED	Valid period:	/
Manufacturer address:	298 Manaia Road, RD5, Masterton 5885, New Zealand		

Note: "/" means this item is not applicable

1.1 Test Article (TA): According to the test requirements, 400mg of the sample was accurately weighed, 3600mg of ultrapure water was added, mixed well, and a sample solution with a maximum test concentration of 10% (w/w) was prepared, and then diluted 1:1, a total of 5 concentrations.

1.2 Sample photo:



TO BE CONTINUED

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2.Control group information

2.1 Positive Control (PC): VC

0.0076g of vitamin C was weighed, the volume was fixed to 15.2mL with ultrapure water, and a stock solution of 0.5mg/mL was prepared, and then diluted sequentially to a total of 5 concentrations.

Chemical Name:	VC
CAS No.:	50-81-7
INCI name:	/
Physical state:	White powder
Purity:	99%
L/N:	J19O10R100374
Relative molecular weight:	176.12
Molecular formula:	$C_6H_8O_6$
Manufacturer:	Shanghai Yuanye Biotech
Valid period:	17th May, 2024

Note: "/" means this item is not applicable

2.2 Positive Control (PC): Ultrapure water

Chemical Name:	Water
CAS No.:	7732-18-5
INCI name:	/
Physical state:	Colorless transparent liquid
Purity:	Class I. pure water
L/N:	20231027
Relative molecular weight:	18.0152
Molecular formula:	H_2O
Manufacturer:	Self-restraint
Valid period:	27th October , 2023

Note: "/" means this item is not applicable

TO BE CONTINUED

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3.Test acceptance criteria

3.1 Experimental principle

The hydroxyl radical detection method is an in vitro model experiment, which produces hydroxyl radicals through the Fenton (H_2O_2/Fe^{2+}) reaction, and the hydroxyl radicals generated by the Fenton reaction of salicylic acid are added to the reaction system to react with salicylic acid to generate 2.3-dihydroxybenzoic acid with special absorption at 510nm. If an analyte with hydroxyl radical scavenging function is added to the reaction system, the hydroxyl radicals generated will be reduced, and the amount of colored compounds will be reduced accordingly.

References:

[1] Wang Xuping, Yang Xiaolan. Study on hydroxyl radical scavenging activity of hop polyphenol extracts with different solvents[J].Journal of Shandong Institute of Light Industry (Natural Science Edition),2013,27(03):20-24.

[2] Guo Penghui, Chen Hong, Ma Jinpu et al. Enzyme-assisted extraction, characterization, and in vitro antioxidant activity of polysaccharides from Potentilla anserina L . [J] . Front Nutr, 2023, 10: 1216572.

[3] Hu T J , Wei X , Zhang X ,et al. Protective effect of Potentilla anserine polysaccharide (PAP) on hydrogen peroxide induced apoptosis in murine splenic lymphocytes[J].Carbohydrate Polymers, 2010, 79(2):356-361.

3.2 Experimental method

CALT/TM/SOP274-01 Standard operating procedure for antioxidant capacity-hydroxyl radical method.

4.Test materials

4.1 Ferrous sulfate solution: take 0.2502g of ferrous sulfate and prepare 90mmol/L ammonium ferrous sulfate solution, and then dilute 10 times.

4.2 Hydrogen peroxide solution: take 0.9926g of hydrogen peroxide with a volume fraction of 3%, set the volume to 10 mL with H_2O, and then dilute 10 times.

4.3 Ethanol-salicylic acid solution: weigh 0.1243g of salicylic acid, dissolve it in ethanol, set the volume to 10 mL with H_2O, and then dilute it 10 times.

5.Test procedures

5.1 Reaction: The sample dosage of each solution is shown in the following table, including sample group, positive control and solvent control (ultrapure water or absolute ethanol), each tube is rated to 15mL, and the reaction is carried out at 37°C in a dark water bath for 15min.

Table 1. Sample table

Reagent	Compose/mL		
	A_0	A_X	A_{X0}
$FeSO_4$	1	1	1
Salicylic acid	1	1	1
PC/TA	/	Amount	Amount
Ultrapure water	12	Amount	Amount
H_2O_2	1	1	/

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5.2 Reading: Absorbance values are determined at a wavelength of 510 nm using a microplate reader.

5.3 Data analysis: Calculate the clearance rate according to the following formula:

$$S=[A_0-(A_X-A_{X0})]/A_0\times100\%$$

S: Clearance rate;

A_0: The absorbance value of the blank control;

A_X: is the absorbance of the test object;

A_{X0}: the control absorbance value of the sample without H_2O_2;

Fitting was performed using GraphPrism to calculate the IC_{50}, the concentration of the solution to be tested at 50% clearance.

6.Analysis

Positive control group	
Concentration（mg/mL）	Clearance（%）
0.0625	81.97±1.41
0.0313	30.35±2.24
0.0156	8.94±0.65
0.0078	7.58±0.98
0.0039	6.86±0.59

Sample group	
Concentration（%）	Clearance（%）
10	82.79±1.27
5	59.31±2.08
2.5	37.90±2.36
1.25	28.01±1.93
0.625	20.75±1.72

Calculation result: Positive control group IC_{50}: 0.04003mg/mL (95%CI：0.03366mg/mL-0.04761mg/mL); Sample group IC_{50}: 6.722% (95%CI：3.423%-13.20%).

7.Conclusion

Under the conditions of this test, the smaller the concentration required for the test substance to achieve half of the scavenging rate, that is, the smaller the IC50, the stronger the ability of the test object to scavenge hydroxyl radicals. At a concentration of 10% (w/w), the scavenging rate of hydroxyl radicals in the sample "STEENS UMF20+ RAW MANUKA HONEY " was 82.79%. The sample "STEENS UMF20+ RAW MANUKA HONEY " has the ability to scavenge hydroxyl radicals. That is, under the conditions of this test, the sample " STEENS UMF20+ RAW MANUKA HONEY " has an antioxidant capacity.

TO BE CONTINUED

TEST REPORT

Test item 2 In vitro anti-aging efficacy assessment

Appendix:

1.Basic sample information

Sample Name:	STEENS UMF20+ RAW MANUKA HONEY	L/N:	/
Chemical Name:	/	Physical state:	Viscous liquid
CAS No.:	/	Colour:	Dark yellow
INCI name:	/	Purity:	/
Product name:	/	Storage Conditions:	Normal temperature
Relative molecular weight:	/	Stability:	Stable under storage conditions
Molecular formula:	/	Production Date:	/
Manufacture:	P. A. & S. C. STEENS LIMITED	Valid period:	/
Manufacturer address:	298 Manaia Road, RD5, Masterton 5885, New Zealand		

Note: "/" means this item is not applicable

1.1 Test Article (TA): According to the test requirements, accurately weigh 0.2007g sample, add 0.8g of ultrapure water, mix, prepare into a 20% (w / w) dilution solution, then a 1:1 dilution, a total of 5 concentrations.

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2.Control group information

2.1 Positive Control (PC): Vc

Scale Vc 0.1000g, add 1 mL of sodium acetate buffer solution to make 100 mg/mL, then dilute 1:1 for 5 concentrations.

Chemical Name:	Vc
CAS No.:	50-81-7
INCI name:	/
Physical state:	White powder
Purity:	99%
L/N:	J19O10R100374
Relative molecular weight:	176.12
Molecular formula:	$C_6H_8O_6$
Manufacturer:	Yuanye Bio-Technology Co.,Ltd
Valid period:	21st March , 2024

Note: "/" means this item is not applicable

3. Test materials

3.1 Main instrument: microplate reader, 96-well plate

3.2 Incubation conditions: 37 ± 1°C

3.3 Main reagents:

3.3.1 Hyaluronidase, purchased from MCKLIN, Lot: C13799004;

3.3.2 Hyaluronic acid, purchased from MCKLIN, Lot: C12393533;

3.3.3 CTAB, purchased from MCKLIN, Lot: C13013956;

3.3.4 Sodium acetate buffer, purchased from Guangzhou chemical reagent factory, Lot: 20211201 17

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4.Test acceptance criteria

4.1 Experimental principle:

Hyaluronic acid is synthesized by the fibroblasts in the dermis. Hyaluronic acid in the skin can reduce the degeneration of collagen and elastin, slow down the degeneration rate from soluble protein to insoluble protein. It also can improve the skin cells colloid solution environment, thus the wrinkles produced by the space between cells can be smoothed out. Ultraviolet light (UV) irradiation can lead to the production of hyaluronidase in the skin, which could degrade hyaluronic acid and lead skin photoaging. Hyaluronic acid is the third key ingredient participating in the skin moisturizing. It can co-effect with natural moisturizing factor (NMF) and skin natural lipid bilayer to promote and maintain the epidermis and dermis moisturizing. Skin barrier injury need to supplement hyaluronic acid, to promote the synthesis of hyaluronic acid and inhibit its degradation, and maintain enough NMF and lipid levels, to keep the skin moisturizing. In this method, the remaining hyaluronic acid after the hydrolysis reaction can react with cetylammonium bromide (CTAB) cationic surfactant to form a complex, resulting in turbidity phenomenon. The inhibition result of the sample on hyaluronidase was detected by colorimetry to evaluate its efficacy.

References:

[1] Pahang. Isolation, purification and properties of Zhoushan cobra venom hyaluronidase [D]. Guangdong Pharmaceutical University, 2016.

[2] Yang Jian'an, Zhang Chao, Wen Yanbing, et al. Carboxylation and acetylation modification of meal polysaccharide and its inhibition of hyaluronidase [J]. Food and Machinery, 2021,10 (37): 44-49.

[3] Ferrante N D. Turbidimetric measurement of acid mucopolusaccharides and hyaluronidase activity[J]. J Biol Chem, 1956, 220:303-306

[4] McDaniel DH, Dover JS, Wortzman M, Nelson DB. In vitro and in vivo evaluation of a moisture treatment cream containing three critical elements of natural skin moisturization. J Cosmet Dermatol. 2020 May;19(5):1121-1128.

4.2 CALT/TM/SOP282-01 Standard operating procedures for the in vitro hyaluronidase inhibition assay.

5.Test procedures

5.1 Reaction:

5.1.1 Blank group(B): 50 μL of hyaluronic acid and 300 μL of sodium acetate buffer solution were added to 1.5 mL EP tubes;

5.1.2 Reaction group(C): 50 μL of hyaluronic acid, 150 μL of sodium acetate buffer solution and 200 μL of hyaluronidase were added to 1.5 mL EP tubes;

5.1.3 Positive control / sample group(A): 50 μL of hyaluronic acid, 50 μL of sodium acetate buffer solution, 200 μL of hyaluronidase, and 100 μL of vitamin C / test sample were added to the 1.5 mL EP tube;

5.1.4 Reactions were performed for 150 min at 37 ± 1°C.

5.2 Microplate read: After the reaction, 500 μL of CTAB solution was added and incubated at 37 ± 1°C for 3min, take 200 μL to 96 well plates, and the absorbance value was measured at 400nm using a microplate reader.

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5.3 Data analysis: The inhibition rate is calculated by the following formula:

$$\text{Inhibition rate}(\%)=(OD_A-OD_B)/(OD_B-OD_C)\times 100$$

Fitting using GraphPrism, calculate IC_{50}, i.e., the concentration of the test sample at an inhibition rate of 50%.

6.Experimental result

Positive control group		Sample group	
Concentration （mg/mL）	Restrain（%）	Concentration（%）	Restrain（%）
100	92.38±2.44	20	51.91±2.59
50	52.89±2.30	10	40.15±1.69
25	21.35±1.20	5	26.14±1.72
12.5	10.79±0.71	2.5	15.76±0.04
6.25	6.88±0.60	1.25	9.38±0.16

Calculation result: Positive control group IC_{50}： 59.67 mg/mL (95%Cl： 49.40-72.07 mg/mL); Sample group IC_{50}:7.76%（95%Cl： 5.12-11.78%）.

7.Conclusion

Under this experimental condition, the smaller the concentration required for the test substance to achieve half of the inhibition rate, that is, the smaller the IC_{50}, the subjects had a stronger ability to inhibit hyaluronidase. At a concentration of 20 % (w/w) , the inhibition rate of hyaluronidase by sample " STEENS UMF20+ RAW MANUKA HONEY " was 51.91%. That is, the sample " STEENS UMF20+ RAW MANUKA HONEY " has inhibitory effect on hyaluronidase. Under the experimental conditions, the sample has an anti-aging capacity.

TO BE CONTINUED

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