

In vitro assessment of antimicrobial activity against *Malassezia furfur* and *Cutibacterium acnes* in relation to anti-ruddy complexion effect of a topical emulsion that contains *Tambourissa trichophylla* leaf extract

I . Objective and outcome of the study

1. Objective

Tree bark of 'Rosabora' (*Tambourissa trichophylla*) as a species endemic to Madagascar at the west of the Indian Ocean has been prescribed for treatments of wound or reddish skin from ancient times, and its palm-size leaf is also known for the antibacterial and the anti-inflammation potency that has been fully utilized for the treatment of stomatitis as well.¹⁾ In this study, we attempted to examine the bactericidal property of a topical emulsion containing Rosabora leaf extract, particularly against *Malassezia furfur*' and *Cutibacterium acnes*' as major resident microbiotas causing skin inflammation and chronic 'rosacea' (so-called 'ruddy face') in consequence.

2. Outcome

Total viable count (TVC) of testing broth cultured in petri plates that inoculated with 1vol% of diluted solution derived from a sanitized vial of 'ULU shake moist milk' with either *Malassezia furfur*' or *Cutibacterium acnes*' added for the activation from 1 to 360mins.

II . Test sample and research methods

1. Test sample

'ULU shake moist milk' (Sold by Japan Dryskin Laboratory Co., Ltd.)

【Ingredients】 Water, Squalane, Trehalose, Ceramide 2, Ceramide 6II,

Ceramide 3, Ceramide 5, Ceramide 1, Hyaluronic Acid,
Tambourissa trichophylla leaf extract, Jojoba Seed Oil, Rosehip
Oil, Phytosterols, Hydrogenated lecithin

2. Research methods

(1) Implementing agency

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(2) Test strains

Table 1.

Scientific Name	NBRC Reg.#
<i>Malassezia furfur</i>	0656
<i>Cutibacterium acnes</i> *	107605

*The name has been updated from '*Propionibacterium acnes*'.

(3) Preparation of test strains

① *M. furfur*

To incubate the test strain at 30°C for 48 hours on Glucose Peptone Agar with Lecithin & Polysorbate (GPLP Agar).

To suspend the precultured strain in sterile saline for preparation of test samples which viable counts were adjusted to 1.0×10^8 /ml for the baseline.

② *C. acnes*

To incubate the test strain at 35°C for 48 hours on Gifu Anaerobic Medium (GAM Agar).

To suspend the precultured strain in sterile saline for preparation of test samples which viable counts were adjusted to 1.0×10^8 /ml for the baseline.

(4) Testing routine

1vol% (0.2ml) of inoculum was added to 19.8g of the test sample (ULU SMM) in a sanitized vial and kept in an incubator which a temperature was set at 25°C. Then, 1g of the test sample was taken out from the vial after 1min, 180mins, and 360mins for dilution with 9mg of LP solution* in a gradual process, and the TVC was measured by agar plate dilution method at the end.

For control strain, the same procedure for preparation, incubation, and measurement was taken toward the test sample filled with sterile purified water.

*LP solution: Polypepton 1g, Egg lecithin 0.7g, Polysorbate80 20g, Purified water 980ml

(5) Culture media and conditions for viable count of each test strain

① *M. furfur* : GPLP Agar medium, 30°C、 3 days

② *C. acnes* : GAM Agar medium, 35°C、 2 days (Anaerobic culture)

3. Results

As a result of this antimicrobial efficacy test, the test samples successfully diminished total viable counts (TVC) for both test strains by four digits after 6 hours of duration against control strains.

Table 2.

Test Strain	Test Sample	TVC /g by Duration of Action			
		0 min.	1 min.	180 min.	360 min.
<i>Malassezia furfur</i>	Control (Sterile purified water)	1.1×10^6	1.0×10^6	1.0×10^6	1.0×10^6
	ULU shake moist milk	-	6.4×10^5	4.2×10^3	2.5×10^2
<i>Cutibacterium acnes</i>	Control (Sterile purified water)	3.1×10^6	3.3×10^6	3.2×10^6	3.4×10^6
	ULU shake moist milk	-	3.2×10^6	1.9×10^3	1.4×10^2

4. Conclusion

Since the result of the study has proven the antibacterial effect of the test sample against '*Malassezia furfur*' and '*Cutibacterium acnes*' as major causes of ruddy face, we conclude that the effect of the test sample on the improvement of

‘ruddy face’ as a synonym for chronic facial skin inflammation has been implied as well.

Moreover, in separate clinical research targeted to evaluate the product safety toward adults who claimed to have an atopic disposition in a 24hr hour-human closed patch testing method (conducted in Oct. 2020), the acquired ‘Stimulation Index’ of the test sample recorded below 0.0 whereas the norm for safety product was 5.0. It also means to us that the topical emulsion containing Rosabora leaf extract has almost no adverse effect to cause any skin inflammation despite its long-hour usage.

[Reference]

- 1) A concept sheet of Ambora extract - ‘Rosabora as the rare species of plants to cure a concerned redness of your skin’ : Matsumoto Trading Co.,Ltd, / Nov. 8, 2019
[<https://matsumoto-trd.com/product/pdf/concept/a09.pdf>]
- 2) ‘A report on 24 hour-human closed patch test targeted to male and female adults who claimed to have an atopic disposition’ (Ref.#200929-001) : DRC Co.,Ltd. / Nov. 5, 2020