Using magnolia bark in multifunctionals

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ABSTRACT

In this study, we investigate the efficiency of *Magnolia officinalis* bark extract and heptyl undecylenate (A-Silkne) in antimicrobial activity and preservative effects. The results of this study demonstrate that A-Silkne has a good preservative effect as a natural-derived antimicrobial agent, and is particularly synergic with various preservatives. In addition, A-Silkne was found to have potential efficacy for anti-acne and similar skin feel to silicone oils. With this study, it is concluded that A-Silkne, as a multifunctional ingredient, can be an excellent alternative, reducing the use of higher doses of chemical/synthetic preservatives that are often associated with sensitivity and allergic reactions.

Preservation of personal care products containing water and organic/inorganic ingredients is necessary to prevent spoilage that subsequently can cause severe health risks for consumers. One of the major concerns in personal care products is product spoilage causing microbial contamination.

Personal care products have a nutrient-rich medium that favours the growth of microorganisms. Microbial contamination may occur while being manufactured or when consumers use the products. Therefore, preservative systems are used in personal care products to prevent microbial spoilage, improve the shelf life of the product, and protect consumers against adverse microbial infection.[1]

One of the most common approaches used is the addition of preservatives such as paraben, which are esters of 4-parahydroxybenzoic acid (PHBA). Formaldehyde releasers, isothiazolinones, triclosan, urea-based preservatives are also commonly used as preservatives in personal care products.

However, the use of popular preservatives has become controversial. Paraben is involved in endocrine disruptors and may make women more prone to developing estrogen positive breast cancer.[2]

Meanwhile, formaldehyde releasers, which are added to shampoo, soaps, nail polishes, hair dyes, and conditioners have side-effects of formadehyde exposure. Formaldehyde is considered as a strong allergen, and contact with this preservative may induce chronic allergic eczema. It has been observed that using skincare products containing a low amount of formaldehyde (2.5 ~ 400 ppm) in people with allergies led to dermatitis.[3]

The debate has been further heightened by the growing popularity of pro-ecological and healthy lifestyle trends. It is not surprising that personal care product formulators have introduced alternative preservatives as safer than paraben.[4]

The following strategies are implemented to efficiently control microbial contamination, and minimise adverse health effects from preservatives: (1) replacement of preservatives with other chemical or natural alternatives; (2) formulation of the mixtures of preservatives with other alternatives; and (3) preparation of the composite mixtures of various alternatives to the preservative-free system. Consequently, preference for 'preservative-free' and 'self-preserving' products is a rising trend among consumers.

Multifunctional ingredients

Multifunctional ingredients are defined as single ingredients that have more than one function in personal care product formulations. Multifunctional ingredients with antimicrobial activity are seen as potential alternatives.

As these ingredients are not designed to be preservatives, they tend to be either antimicrobial or preservative boosters. These types of ingredients can help formulators reduce chemical inventory, reduce formula complications, and improve ease of manufacture. In recent years, therefore, formulators have taken a greater interest in multifunctional ingredients.

Natural extracts could be used as alternative preservatives as they have various properties in personal care products, including anti-inflammatory, antioxidant and anti-microbial properties. In particular, the anti-microbial activity of natural extracts is more effective in inhibiting the growth of microorganisms due to various modes of action.[5]

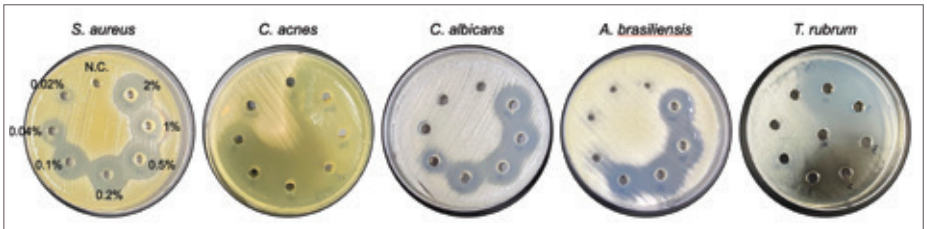
Therefore, we conducted research to develop plant-derived *Magnolia officinalis* bark extract and heptyl undecylenate as multifunctional ingredients with various activities.

Antimicrobial activity of *Magnolia officinalis* bark extract

Magnolia officinalis bark extract is an important herb that contains diverse, biologically active compounds, including magnolol and honokiol. To evaluate the anti-microbial activity, a well-diffusion assay was performed. The degree of anti-microbial activity was shown by measuring the diameter of the inhibition zone formed on the plate (Figure 1).

According to the analysis results, *Magnolia officinalis* bark extract was strongly antimicrobial and antifungal activity against skin-related microorganisms, such as *Staphylococcus aureus, Cutibacterium acnes, Candida albicans, Aspergillus brasiliensis,* and *Trichophyton rubrum*. Concentrations greater than or equal to 0.1% of *Magnolia officinalis* bark extract completely inhibited the growth of gram-positive bacteria, yeast and mould, *S. aureus* and *C. albicans*, whereas greater than or equal to 0.02% of *Magnolia officinalis* bark extract inhibited the growth.

However, it did not show an inhibitory effect on gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). As a result, *Magnolia officinalis* bark extract can potentially be used as an antimicrobial agent for personal care products.



INHIBITION ZONE SIZE (MM)

Magnolia offcinalis bark extract conc.	Bacteria Gram (+)		Yeast	Mould	
	S. aureus	*C. acnes*	*C. albicans*	*A. brasiliensis*	*T. rubrum*
2%	22	>30	19	20	30
1%	22	>30	19	20	30
0.5%	22	>30	18	20	20
0.2%	21	>30	16	17	20
0.1%	18	>30	10	8	15
0.04%	11	18	8	-	-
0.02%	8	-	7	-	-

N.C. : DMSO
Well size: 6mm
-: No antimicrobial activity

Figure 1: Antimicrobial analysis of *Magnolia officinalis* bark extract

Preservative boosting properties of A-Silkne in formulation

Natural preservatives derived from plant and microorganisms with antimicrobial activity can reduce or eliminate the use of preservatives in personal care product formulations.
The preservative efficacy test (PET), also known as the challenge test, determines the effectiveness of the antimicrobial agent by calculating the logarithm of the number of viable microorganisms after microbial contamination of the product.

In this study, the PET was made using Personal Care Products Council guidelines. A combination of *Magnolia officinalis* bark extract and heptyl undecylenate (A-Silkne) was used in an oil balm to evaluate the preservative efficacy.

Microorganisms used for artificial contamination were bacteria (*E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6038), yeast (*C. albicans* ATCC 10231) and mould (*A. brasiliensis* ATCC 16404) at a final concentration of 10^6 CFU/g for bacteria and 10^5 CFU/g for yeast and mould. The preservative efficacy was determined by sampling 1g from the formulation at each time-point of 0, 7, 14, and 28 days.

In the PET with 1% A-Silkne, there was an immediate reduction of bacteria growth after seven days and inhibited the growth of bacteria until 28 days (Table 1). Although the inhibitory effect on yeast and mould was weak, the inoculated microorganisms steadily decreased for four weeks. Therefore, A-Silkne can be used as a preservative booster for effective preservative systems of personal care products.

Preservative effect of A-Silkne in combination with glyceryl caprylate

To evaluate the preservative effect in various formulations, PET was conducted by combining A-Silkne with glyceryl caprylate, a natural multifunctional ingredients. Specifically, in order to evaluate the preservative efficacy of the A-Silkne with glyceryl caprylate in a various personal care products formula (oil balm, O/W type cream, and shampoo), samples were inoculated separately with each microorganism at a final concentration of 10^6 CFU/g for bacteria and 10^5 CFU/g for yeast and mould.

The preservative efficacy was determined by sampling 1 g from the formulation at each time-point of 0, 7, 14, and 28 days. In a formula of oil balm preserved with 2% A-Silkne and 0.4% glyceryl caprylate, from day 7, bacteria (*E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6038), yeast (*C. albicans* ATCC 10231) and mould (*A. brasiliensis* ATCC 16404) were not detectable.

THE NUMBER OF VIABLE MICROORGANISMS (LOG CFU/g)

	A-Silkne conc. (%, w/w)	Day 0 (inoculation)	Day 7	Day 14	Day 28
Bacteria	1%	6.3	0	0	0
	2%	6.3	0	0	0
	3%	6.3	0	0	0
Yeast	1%	5.4	5.4	5.4	5.3
	2%	5.4	5.4	5.3	5.2
	3%	5.4	5.3	5.2	5.1
Mould	1%	5.3	5.2	5.0	5.0
	2%	5.3	5.1	5.0	5.0
	3%	5.3	5.1	5.0	4.9

Table 1: Preservative efficacy test of A-Silkne in oil balm

THE NUMBER OF VIABLE MICROORGANISMS (LOG CFU/g)

		Day 0 (inoculation)	Day 7	Day 14	Day 28
Oil balm	Bacteria	6.3	0	0	0
	Yeast	5.2	0	0	0
	Mould	5.3	0	0	0
Cream (o/w type)	Bacteria	6.3	1.0	0	0
	Yeast	5.2	2.7	0	0
	Mould	5.3	4.3	4.2	3.4
Cream (w/s type)	Bacteria	6.6	0	0	0
	Yeast	5.3	2.1	0	0
	Mould	5.0	2.3	1.3	0
Shampoo	Bacteria	6.3	0	0	0
	Yeast	5.2	0	0	0
	Mould	5.3	4.2	4.0	3.0

Table 2: Preservative efficacy test of A-Silkne with glyceryl caprylate in various formula

In a formula of cream (O/W type) preserved with 2% A-Silkne and 0.4% glyceryl caprylate, from day 14, bacteria and yeast were not detectable. From day 7 to day 28, mould was gradually decreased. In a formula of shampoo solution preserved with 2% A-Silkne and 0.4% glyceryl caprylate, from day 7, bacteria and yeast were not detectable.

From day 7 to day 28, mould was gradually decreased (Table 2). This suggests that using a 2% A-Silkne with 0.4% glyceryl caprylate shows an excellent preservative effect on various personal care products. This revealed that a formulation A-Silkne with a natural alternative preservative was effective against all microbe containing fungi. This also demonstrated that A-Silkne could be applied to the creation of new preservative-free system for use in the personal care product industry.

Multifunctional benefits – potential of anti-acne agents

Acne is a chronic inflammatory disease outbreak in the sebaceous glands within the hair follicle. The proliferation of *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) causes monocytes to stimulate the secretion of inflammatory cytokines. *C. acnes* hydrolyses neutral lipids into free fatty acids, which promotes oxidative stress, inflammatory reaction, and tissue distruction.

C. acnes also interacts with various components of the immune system. In addition, the aerobic bacteria *Staphylococcus aureus* proliferates in acne lesions and causes inflammatory skin diseases. Usually, salicylic acid and *Centella asiatica* extract including madecassoside are used to treat *C. acnes*. Salicylic acid and *Centella asiatica* extract can also regulate the proliferation of *C. acnes*, but they can cause several side effects including dry skin.[6]

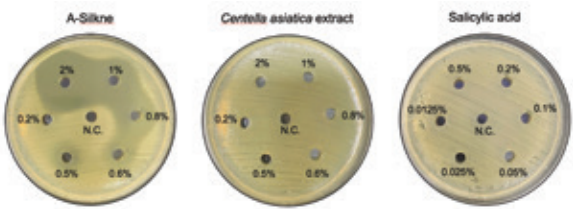
Therefore, the antibacterial activity of salicylic acid and *Centella asiatica* extract, commonly used ingredients for acne treatment, as compared with A-Silkne. To evaluate the anti-microbial activity of *C. acnes* (*C. acnes* ATCC 6919), a well diffusion assay was performed. The degree of anti-microbial activity was shown by measuring the diameter of the inhibition zone formed on the plate (Figure 2).

In this study, the concentrations of salicylic acid, *Centella asiatica* extract, and A-Silkne required to inhibit cell growth of *C. acnes* were >0.5%, >2%, and 0.6%, respectively. Salicylic acid, which is generally used less than 0.5% due to skin dryness and irritation as an ingredient in personal care products for acne treatment, showed no antibacterial effect at 0.5% concentration.

In addition, the antibacterial activity of *Centella asiatica* extract was not confirmed against *C. acnes*. On the other hand, A-Silkne was inhibited the cell growth of *C. acnes* at 0.6% concentration. In this study, A-Silkne was able to effectively inhibit *C. acnes* at a low concentration without causing side effects such as skin dryness or skin irritation.

We evaluated the growth inhibitory effect of A-Silkne on *C. acnes* in oil balm (Figure 3). For analysis, *C. acnes* was contaminated with more than 10^6 CFU/g, and viable *C. acnes* was accumulated on days 1, 2, 3, 7, 14, 21, and 28 (Figure 2). As a result, *C. acne*, which was contaminated in the oil balm, was inhibited by 99.999% after two days.

We showed that A-Silkne has excellent antibacterial activity against *C. acnes* as well as a powerful protect ability against contaminated *C. acnes*. According to analysis results, it is expected that effective acne care products can be developed by applying A-Silkne to the products.



INHIBITION ZONE SIZE (MM)

Sample conc. (%, w/w)	A-Silkne	*Centella asiatica* extract
2%	32	-
1%	22	-
0.8%	18	-
0.6%	10	-
0.5%	-	-
0.2%	-	-

INHIBITION ZONE SIZE (MM)

Sample conc. (%, w/w)	Salicylic acid
0.5%	-
0.2%	-
0.1%	-
0.05%	-
0.025%	-
0.0125%	-

N.C. : DMSO
Well size: 6mm
-: No antimicrobial activity

Figure 2: Antimicrobial analysis of A-Silkne against *C. acnes*

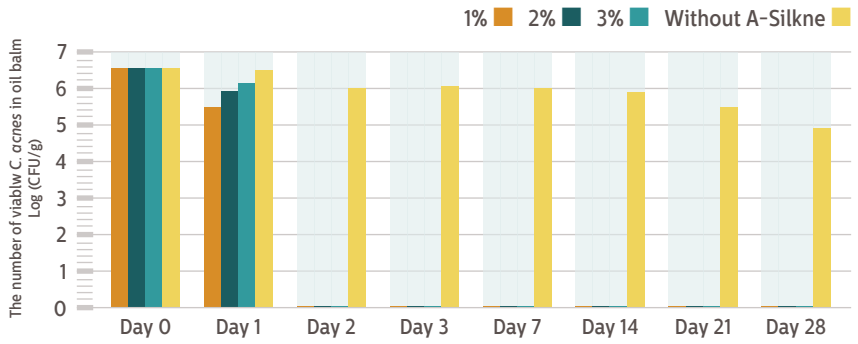
Multifunctional benefits – potential of alternative silicone oils

Silicone oil is one of the most widely used ingredients in personal care products. Silicone oils, such as dimethicone, offer excellent spreading properties and give end-use products a soft, pleasant and non-sticky after-feel.

Silicones have exceptional physicochemical and sensory properties but their high chemical stability results in very low biodegradability. Consumers are becoming increasingly sensitive to environmental issues and demand more environmentally friendly personal care products. This recognition strongly encourages the personal care products industries to develop plant derived biodegradable alternatives to silicone oils.[7]

We conducted a blind sensory test to compare the spreadability, absorption, moisturising, film formation, and feeling of A-Silkne and silicone oil (Figure 4). As a result, spreadability, absorption, moisturizing and film formation were improved compared to placebo oil balm.

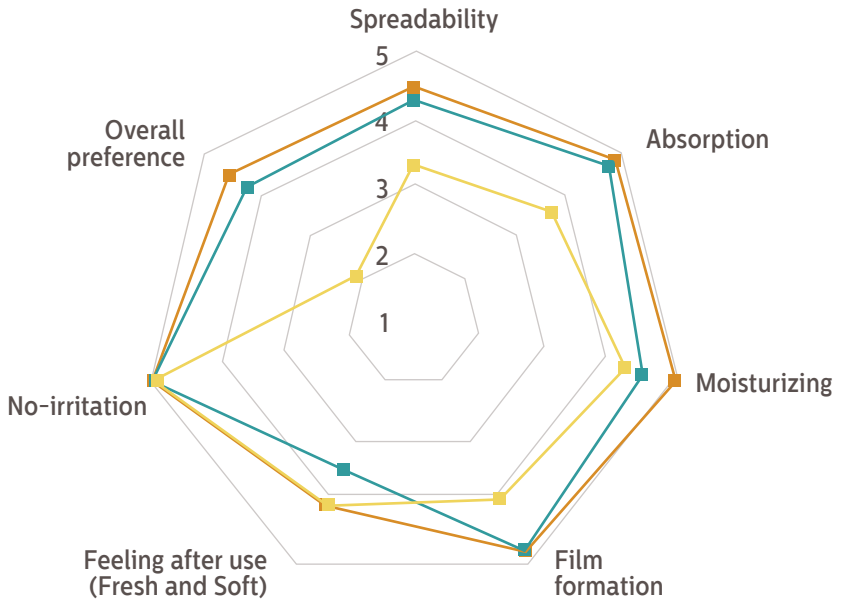
In addition, a similar level of feeling after



THE NUMBER OF VIABLE *C.ACNES* IN OIL BALM LOG REDUCTION (CFU/g)

Sample conc. (%, w/w)		Day 0 (inoculation)	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
A-Silkne Conc. (%, w/w)	1%	6.5	5.5	0	0	0	0	0	0
	2%	6.5	5.9	0	0	0	0	0	0
	3%	6.5	6.1	0	0	0	0	0	0
Without A-Silkne		6.5	6.4	6.0	6.1	6.0	5.8	5.5	4.9

Figure 3: Analysis of growth inhibitory effect of A-Silkne on *C. acnes* in oil balm



- Sample: Oil balm
- Volunteer: Aged 20-50, men/women, 10 people
- Method : A questionnaire survery performed after applying it onto the back of bands

	5 points	~	1 points
Spreadability	Very Good	~	Very Bad
Absorption	Very Good	~	Very Bad
Moisturizing	Very Good	~	Very Bad
Film formation	Very Good	~	Very Bad
Feeling after use	Fresh and Soft	~	Sticky
Irritation	No-irritant	~	Irritating
Overall preference	Very Good	~	Very Bad

Figure 4: Blind sensory test of A-Silkne

use was confirmed as compared to the same amount of silicone oil (dimethicone). In the sensory comparison analysis, A-Silkne showed performance attributes required for enhancing the sensory profile of oil balm formulation. Therefore, these sensory results show that A-Silkne can be used as plant-derived biodegradable ingredient to replace synthetic silicone oil.

Safety of A-Silkne

To verify the good cutaneous compatibility of the investigational A-Silkne, after single application, patch test was conducted on 33 adult testees aged 20-60s.[8] The application of the investigational A-Silkne was performed on the back - between the hips and the shoulders - free from any macroscopic trace of irritation or from any abnormality (scars, moles, freckles), which could interfere with the interpretation of the results. The patches were applied to the back of the subjects for 24 hours.

Grading was performed at 30 minutes and 24 hours after patch removal, to the naked eye and a magnifying glass. For data analysis, the calculation of the primary cutaneous irritation (PCI) index, calculated for 30 minutes and 24 hours allowed for determining the A-Silkne compatibility with the skin (Figure 5).

As a result, A-Silkne was a no-irritant and safe ingredient even at a concentration of 100%. Consequently, A-Silkne was very low irritation compared to the commonly used preservative boosting ingredients such as 1,2-alkanediol or acne treat ingredients such as salicylic acid.

Conclusion

In conclusion, the study found A-Silkne inhibits the growth of microorganisms, including fungi and acne-causing bacteria. Therefore A-Silkne can be recommended as a candidate for effective an alternative preservative system. This shows that A-Silkne can be simultaneously replaced the use of synthetic preservatives or silicone oils.

In addition, it was analyzed by sensory test and patch test which leads to an excellent feeling-after-use and no-irritation, respectively. These interesting results demonstrate that A-Silkne is a new multifunctional ingredient for personal care products that is effective as an anti-microbial, and this can be feasible to resolve the sensitive skin issues occurring by the mask regularly used in COVID-19 situations. **PC**

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		P.C.I	Conclusion
A-Silkne Conc. (%, w/w)	2% (in squalene)	0.00	No-irritant
	10% (in squalene)	0.00	No-irritant
	100%	0.02	No-irritant
Squalene		0.00	No-irritant

The P.C.I. was calculated according to the following formula:

$$P.C.I. = \frac{\Sigma \text{ Grade of each subject}}{\text{Number of subjects x number of readings}}$$

P.C.I	Application
0 ≤ P.C.I. ≤0.05	No-irritant
0.05 < P.C.I. ≤0.15	Slight irritant
0.15 < P.C.I. ≤0.5	Moderate irritant
> 0.5	Strong irritant

Figure 5: Safety of A-Silkne