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Effects of the myrtle essential oil on the acne skin—clinical trials for Korean women

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Abstract

Background: This study aims to clinically verify the effects of myrtle essential oil, which has non-irritation, non-toxicity, and non-sensitivity characteristics among natural materials, by applying the oil to acne skin of Korean women, with special emphasis on a development of natural substances with a high safety to relieve acne and minimize skin irritation.

Methods: After a homogeneity test was conducted targeting 20 Korean women with acne skin, who have met the primary and the secondary clinical selection criteria, the subjects were divided into the treatment group with myrtle essential oil applied (the experimental group) and the no-treatment group with no myrtle essential oil applied (the control group) to use provided cosmetics every morning and evening for 6 weeks. To precisely check the effects of the myrtle substances, the provided cosmetics are made with only a difference in the presence or absence of added myrtle substances, leaving all other substances equal.

Results: The acne grades significantly decreased in the experimental group with myrtle applied, with the pore index (outstanding pores, large pores, blackheads), the erythema index, the sebum index, and the desquamation index also decreased in the group. Lastly, the microorganism index decreased considerably in the experimental group, showing all evaluation indicators related to acne improved in the experimental group in a statistically significant manner. On the other hand, the acne grades a little decreased in the control group with no myrtle applied, but with no statistical significance, while the pore index (outstanding pores, large pores, blackheads), the erythema index, the sebum index, and the desquamation index rather increased to some extent. In addition, the microorganism index decreased in the control group, but with a smaller volume of changes than that in the experimental group.

Conclusion: This study has clinically proved that myrtle essential oil has effects for convergence, reduction in erythema, removal of sebum and dead skin cells, and antibacterial activity on the facial skin of Korean women. Especially, this study confirmed that myrtle essential oil is a safe, skin-soothing substance effective for treating acne by showing the oil reduced the number of erythema.

Trial registration: -Name of the registry: Institutional Review Board of Korea Institute of Dermatological Sciences -Trial registration number 1-70005239-AB-N-01-2018-KIDS-AHA043-HR-01 -Date of registration: March 2, 2018.

Keywords: Myrtle, Acne, Pores, Erythema, Sebum, Dead skin cells, Antibacterial

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Background

Acne is made by a combination of an increased sebum in follicles, hyperkeratinization of dead skin cells, proliferation of *P. acne*, inflammatory reaction, etc. (Sandoval et al. 2014). Treatment substances currently applied to acne show rapid effects, but still many issues are being reported, including erythema, occurrence of resistant strains due to antibiotics, teratogenicity, pruritus, pyrosis, and hormonal side effects (Khunger et al. 2008). To settle those issues, therapies are being presented for acne using glycyrrhizic acid, ginnarin, astemisic acid, berberine, hesperidin, etc., which are obtained from natural materials (Momin et al. 2010; Del Rosso et al. 2016; Boni and Nehrhoff 2003). Among various natural substances, essential oil has been used as attractive materials for cosmetics in the American and European markets over the past decades, having also been used for home remedy across the world already for centuries (Yang et al. 2004). Myrtle essential oil has been used for treating acne, oily skin, and loose pores especially around the Mediterranean even from the Middle Ages (Li et al. 2018; Battaglia 1997), being known as a soft, mild sort of oil with effects for reproduction, convergence, and prevention of allergy (Aleksic and Knezevic 2014). Myrtle essential oil comprises 35.9% of myrtenyl acetate, an ester substance; 29.89% 1,8-cineole, an oxide substance; and 8.18% α-pinene and 7.58% of limonene, which are mono terpene substances (Battaglia 1997). These ester, oxide, and mono terpene-based substances present effects for convergence, soothing, prevention of germs, and reduction of dead skin cells (Ghasemi et al. 2011; Battaglia 1997). Especially, while most substances with effects for reduction of dead skin cells and sebum and prevention of germs make the human skin more sensitive, myrtle, thanks to its 36% of ester substances, is reported to treat acne even without irritating the skin (Battaglia 1997). There is also a great deal of other literature on the effects and applications of myrtle, but any data verifying those effects directly through a clinical test have not been found anywhere. Accordingly, this study aims to examine the impacts of myrtle essential oil on Koreans' acne skin and to reveal its possibility to be used for cosmetics for acne skin with a high safety, which minimizes irritation to the skin without causing allergic reactions.

Methods

Subjects and study design

From among Korean women with acne on their facial skin, this study selected 20 subjects meeting the clinical selection criteria, excluding those who are not

appropriate for the clinical test through a primary survey. The selection criteria are as follows:

- Those who have neither atopy nor chronic skin disease:
- Those who are not having their acne treated currently;
- Those who have not had their facial skin peeled over the past 6 months;
- Those who have not received skin scaling, laser treatment, acne treatment, or skin care for acne skin to treat acne over the past 1 month;
- Those who have not taken a medicine which may affect the hormonal system over the past 1 month;
- Those who have not used any acne treatment medicine or cosmetics including AHA, salicylic acid, and sulfa over the past 1 month;
- Those who do not have any allergy to cosmetics; and,
- Those who have understood the objective of this study and have given their consent to take part in the study.

By conducting a homogeneity test for each of the Korean Acne Grading System (KAGS), the erythema index, the pore index, the sebum index, the desquamation index, and the microorganism index, this study selected 10 persons for the experimental group and 10 other persons for the control group, who are statistically equal, as the final subjects for the tests. This study conducted three sessions of self-skin care training and the clinical test for 6 weeks from 5 March to 16 April 2018. The subjects were informed about all information with regard to the test and took part in the test after signing a written consent according to their will. In addition, all clinical tests for this study have undergone the deliberation of the Institutional Review Boards before starting the tests 1-70005239-AB-N-01-2018-KIDS-AHA043-HR-01).

Test method

The control group was used a foam cleanser, toner, emulsion, and sunblock of a cosmetics company with the initial of R every day, any of which does not contain myrtle substances, and a cream pack once a week, as a skin care on their own. The control group received a salon treatment once a week, which includes the steps of pressing out acne; applying an algae mask with no myrtle substances for 15 min; and applying a toner, emulsion, and sunblock made by the cosmetics company R, which do not contain any myrtle substances. On the other hand, the experimental group was let use a foam cleanser, toner, emulsion, and sunblock made by the cosmetics company R every day, all of which contain myrtle substances, and a cream pack containing myrtle

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substances once a week, as a skin care on their own. The experimental group also received a salon treatment once a week, which includes the steps of pressing out acne; applying an algae mask with myrtle substances for 15 min; and applying a toner, emulsion, and sunblock made by the cosmetics company R, all of which contain myrtle substances.

Measurement method

Measurement of changes by KAGS

The Korean Acne Grading System (KAGS) is an evaluation system reflecting the characteristics of acne on the skin of Koreans, which classifies the severity of acne into six grades, reflecting the dynamic clinical features of Koreans' facial skin (Table 1). The KAGS present the reference photographs of Koreans for each grade and the scope-type criteria for reference lesions, thereby seeking application convenience, clarity, and objectivity in Koreans to be available for determination of treatment effects. According to the KAGS criteria, this study observed and compared the changes in severity in the control group and the experimental group, and determined the acne grades of the subjects for this study before, 3 weeks after, and 6 weeks after using the provided cosmetics products.

Measuring devices

To measure the erythema index (EI) of the acne skin, this study used the Mexameter MX18 (Courage and Kazaka Electronic, Germany). To measure the pore index, this study filmed the pore condition of the subjects' faces by using the RSA (Robo skin analyzer CS50, Japan) and analyzed the pore index by using a program attached to the RSA. To measure the sebum index, this study attached a measuring tape with the same pressure, detached the tape after the same period of time, and measured the quantity of sebum by using an oil and moisture measuring device (Skin diagono system, Bomtech, Korea). To measure the desquamation index (DI) of the surface of the skin, this study attached a measuring tape with the same pressure, detached the tape after the same period of time, and figured out the

Table 1 Korean acne grading system

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Grade	Description
1	Papules ^a ≤ 10
2	Papules 11–30
3	Papules ≥ 31, nodules ^b ≤ 10
4	Nodules 11–20, \pm mild ongoing scars
5	Nodules 21–30, \pm moderate ongoing scars
6	Nodules ≥ 31, ± severe ongoing scars, ± sinus tract

^aPapule, acne which is smaller than 5 mm

desquamation index based on the number of dead skin cells fallen off from the surface of the skin by using the Skin-Visiometer SV600 (CCD camera type-Visioscan). To measure the quantity of microorganisms on the surface of the skin, this study rubbed the forehead, chin, and both cheeks of the subjects with a disposable cotton swab named LuciPac W* seven times for each part, put the end of the cotton swabs in a solution, shook around 10 times, and then measured the relative light unit (RLU) by using the Lumitester TM PD-10 (KIKOMAN, Japan). You can check the presence or absence of microorganisms in accordance with the RLU.

Conditions and parts for measurement

The subjects washed their faces by using the same cleanser, took rest for 30 min (the erythema index, the pore index), 40 min (the sebum index), and 50 min (the desquamation index) under the conditions of constant temperature and humidity (22–24 °C of temperatures, 40–60% of relative humidity), and then had their skin condition measured before, 3 weeks after, and 6 weeks after using the provided products. The measurement was conducted four times with the same posture at the same time zone in the same place under the same lightings. Then, the averages and standard deviations of the measurements were recorded.

The measured parts were the forehead, both cheeks, and the chin. Of the forehead measured was the central point between two acupuncture points of "Shinjung" and "Indang." In the chin, the acupuncture point named "Seungjang" under the lower lip was measured. In both cheeks, the parts vertically down from the center of the pupils, being horizontal with the end of the nose, were the measured points. The average measurements were calculated for the forehead, cheeks, and chin, being used for figuring out the erythema index, the sebum index, and the desquamation index for the entire face.

Test materials

The myrtle essential oil for the test is extracted by a steam distillation method. This study made and used the foam cleanser, toner, emulsion, and cream pack for the test by the below methods.

Foam cleanser

Phase A was dissolved at 65–70 °C, with phase B dissolved at 70–75 °C. Then, cocamidopropyl betaine was put into phase B at a room temperature to dissolve. After that, both phases A and B were saponified at 70–75 °C by a homo mixer at 3500 rpm and a paddle mixer at 20 rpm for 10 min. Cocamidopropyl betaine was put into the saponified phases A and B to mix them by a homo mixer at 3500 rpm and a paddle mixer at 20 rpm for 3 min. The mixture was cooled down to 30 °C, and

^bNodule, acne which is larger than 5 mm

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Myrtus communis extract was finally added only to the cosmetics for the experimental group (Table 2).

Toner

Phase A was dissolved at 45–48 °C, with phase B dispersed for 30 min. They were agitated by a homo mixer at 2000 rpm and a paddle mixer at 25 rpm at 45–48 °C for 10 min. During the agitation process, phase C, which had been dissolved at 45–48 °C, was added. After that, *Myrtus communis* extract was added only to the cosmetics for the experimental group. Phase D was dispersed at a room temperature. Then, phases A, B, and C, which had been mixed with each other, were agitated by a homo mixer at 2500 rpm and a paddle mixer at 25 rpm at 45–48 °C for 5 min. Phase D was added to the mixture during the agitation process. After the agitation, the mixture was cooled down to 28 °C to be filtered and discharged (Table 3).

Emulsion

Phase A was dissolved at 75–78 °C, with phases B and C dispersed for 30 min. Then, phases B and C were put into phase A to be mixed with each other. After that,

Table 2 Formulations of cleansing form ingredients

Ingredients	Compositions	(wt%)
	Control	Myrtle
Phase A		
Water	q.s to 100	q.s to 100
Glycerin	6.00	6.00
Propylene glycol	5.00	5.00
PEG-8	2.00	2.00
Methylparaben	0.20	0.20
Tetrasodium EDTA	0.05	0.05
Potassium hydroxide	5.00	5.00
Cocamidopropyl betaine	0.50	0.50
Phase B		
Stearic acid	5.00	5.00
Myristic acid	20.00	20.00
Lauric acid	5.00	5.00
Beeswax	1.00	1.00
Glycol stearate	2.00	2.00
Propylparaben	0.10	0.10
Hydrogenated polydecene	1.00	1.00
Glyceryl stearate, PEG-100 stearate	2.50	2.50
Cetearyl alcohol	0.50	0.50
PEG-40 hydrogenated castor oil	0.80	0.80
Phase C		
Sodium methyl cocoyl taurate	2.00	2.00
Myrtus communis extract	-	0.50

Table 3 Formulations of toner ingredients

Ingredients	Compositio	ns (wt%)
	Control	Myrtle
Phase A		
Water	q.s to 100	q.s to 100
Trisodium EDTA	0.03	0.03
Betaine	0.70	0.70
Sodium hyaluronate	3.50	3.50
Glycerin	6.00	6.00
Dipropylene glycol	6.00	6.00
Phase B		
Water	12.00	12.00
Acrylates/C10-30 alkyl acrylate crosspolymer	0.12	0.12
Phase C		
SD alcohol 40-B	1.00	1.00
Ehylparaben	0.10	0.10
Methylparaben	0.20	0.20
Triethylhexanoin	1.50	1.50
Pentaerythrityl-tetraethylhexanoate	2.00	2.00
Myrtus communis extract	-	0.50
Phase D		
Water	1.00	1.00
Triethanolamine	0.20	0.20

phase D was dissolved at 75–78 °C, adding cyclopentasiloxane in the middle of the process to be mixed with each other. The mixed phases A, B, C, and D were agitated by a homo mixer at 3500 rpm and a paddle mixer at 25 rpm at 75–78 °C for 5 min. When the agitation is almost finished, water and trethanolamine were put into the mixture. *Myrtus communis* extract was put into the cosmetics for the experimental group, to be agitated by a homo mixer at 3500 rpm and a paddle mixer at 25 rpm at a room temperature for 2 min (Table 4).

Cream pack

Phase A was dissolved at 75–78 °C. Phases B and C, which are aqueous carbomer solution dispersed for 30 min, were put into phase A to be agitated by a homo mixer at 3500 rpm and a paddle mixer at 24 rpm at 75–78 °C for 5 min. Phase D was dissolved at 75–78 °C, adding cyclopentasiloxane to that in the middle of the process. The mixed phases A, B, C, and D were agitated by a homo mixer at 3500 rpm and a paddle mixer at 25 rpm at 75–78 °C for 5 min. Water and triethanolamine were dispersed at a room temperature to be mixed with the agitated phases A, B, C, and D. The whole mixture was agitated by a homo mixer at 3500 rpm and a paddle mixer at 25 rpm at 40–45 °C for 2 min. *Myrtus*

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Table 4 Formulations of emulsion ingredients

Ingredients	Compositions (wt%)	
	Control	Myrtle
Phase A		
Water	q.s to 100	q.s to 100
Trisodium EDTA	0.012	0.012
Methylparaben	0.20	0.20
Betaine	0.50	0.50
Panthenol	0.70	0.70
Glycerin	8.00	8.00
Dipropylene glycol	10.00	10.00
Phase B		
Water	10.00	10.00
Xanthan gum	0.10	0.10
Phase C		
Water	10.00	10.00
Carbomer	0.10	0.10
Phase D		
Cetearyl alcohol	1.50	1.50
Glyceryl stearate	1.00	1.00
Glyceryl stearate, PEG-100 stearate	1.00	1.00
Ehylparaben	0.10	0.10
Propylparaben	0.10	0.10
Macadamia ternifolia seed oil	2.00	2.00
Phytosteryl/isostearyl/cetyl/stearyl/behenyl dimer dilinoleate	0.10	0.10
Hydrogenated polydecene	2.00	2.00
Neopentyl glycol dicaprate	1.00	1.00
Dimethicone	0.30	0.30
Cyclopentasiloxane	1.00	1.00
Water	1.00	1.00
Triethanolamine	0.14	0.14
Myrtus communis extract	_	0.50

communis extract was added only to the cosmetics for the experimental group (Table 5).

Statistical analysis

This study analyzed the statistical data by using the SPSS 12.0, and the characteristics of the subjects were analyzed through a frequency analysis. To examine the differences in effects between the group treated with myrtle and the no-treatment group conducted were the t test and the one-way ANOVA.

Results

Homogeneity test

Acne grades of subjects

In terms of the acne grades, the experimental group consisted of five persons with grade 1 (50%), three

persons with grade 2 (30%), one person with grade 3 (10%), and one person with grade 4 (10%). The control group consisted of six persons with grade 1 (60%), two persons with grade 2 (20%), and two persons with grade 3 (20%). Since there was no statistically significant difference between the two groups, it can be said that this study secured the homogeneity between subjects in acne determination (Table 6).

Skin condition of subjects

To evaluate the skin condition of the subjects, this study measured the erythema index, the number of outstanding pores, the number of large pores, blackheads, the sebum index, the desquamation index, and the microorganism index, but no statistically significant difference was found. Accordingly, it was learned that this study Kim et al. Biomedical Dermatology (2018) 2:28 Page 6 of 10

Table 5 Formulations of cream pack ingredient

Ingredient	Composition	ı (wt%)
	Control	Myrtle
Phase A		
Water	q.s to 100	q.s to 100
Trisodium EDTA	0.10	0.10
Methylparaben	0.20	0.20
1,2-Hexanediol	1.00	1.00
Glycerin	8.00	8.00
Phase B		
Water	10.00	10.00
Carbomer	0.15	0.15
Phase C		
Butylene glycol	8.00	8.00
Sodium polyacrylate	0.25	0.25
Water	20.00	20.00
Phase D		
C14-22 alcohols, C12-20 alkyl glucoside	1.00	1.00
Glyceryl stearate SE	1.00	1.00
Cetearyl alcohol	1.00	1.00
Glyceryl stearate, PEG-100 stearate	1.00	1.00
Butyrospermum parkii (shea butter)	1.00	1.00
Triethylhexanoin	2.00	2.00
Hydrogenated polydecene	5.00	5.00
Caprylic/capric triglyceride	5.00	5.00
Propylparaben	0.05	0.05
Ehylparaben	0.10	0.10
Sorbitan stearate	0.50	0.50
PEG-40 stearate	0.50	0.50
Dimethicone	0.40	0.40
Cyclopentasiloxane	1.00	1.00
Water	1.00	1.00
Triethanolamine	1.00	1.00
Myrtus communis extract	=	0.5

Table 6 Test of homogeneity for Korean acne grading index

KAGS (grade)	Myrtle group N(%)	Control group N(%)	t	р
1	5(50)	6(60)	0.130	0.722
2	3(30)	2(20)		
3	1(10)	2(20)		
4	1(10)	0(0)		

Table 7 Test of homogeneity for skin condition index

	Myrtle group M(± SD)	Control group M(± SD)	t	р
Erythema	392.50(± 62.54)	378.30(± 47.86)	0.479	0.498
Outstanding pore	1271.94(± 677.33)	1127.65(± 905.94)	0.082	0.778
Large pore	38.80(± 46.38)	30.86(± 53.98)	0.041	0.842
Blackhead	649.16(± 468.21)	569.50(± 630.08)	0.049	0.827
Sebum	7.57(± 2.73)	8.66(± 5.44)	2.503	0.131
Desquamation	245.22(± 95.22)	232.49(± 101.45)	0.124	0.729
Microorganism	8343.90(± 3486.55)	7883.30(± 2192.81)	1.374	0.256

secured the homogeneity in skin condition between the subjects (Table 7).

Effects for treating acne skin Findings based on KAGS

In terms of changes in the acne grades due to the treatment using myrtle or no such treatment, the average acne grade in the experimental group significantly decreased from grade 1.8 before the treatment to grade 0.9 (t = 5.014, p = 0.001). In the control group, the average acne grade decreased a little from grade 1.6 to grade 1.5. However, the changes were not statistically significant (t = 0.557, p = 0.591). In terms of changes in the acne grades based on the KAGS in each group, the experimental group treated with myrtle, which had consisted of seven persons with grade 1, three persons with grade 2, one person with grade 3, and one person with grade 4 before the tests, was found 6 weeks after the tests to consist of three persons with grade 0, seven persons with grade 1, 0 persons with grade 2, and one person with grade 3, showing a statistically significant decrease in acne grades of 80% of the subjects (p < 0.01). The control group with no treatment using myrtle, which had consisted of six persons with grade 1, two persons with grade 2, and two persons with grade 3, was found 6 weeks after the tests to consist of six persons with grade 1, three persons with grade 2, and one person with grade 3, showing no particular difference from before the tests. As a result of test of the changes in acne grades between the two groups, it is found there is a statistically significant difference between the two groups with t = 3.151 and p = 0.006 (Table 8).

Changes in pore index

For an analysis of the pore index, the pores on the facial skin of the subjects were divided into outstanding pores, large pores, and blackheads. The outstanding pore index decreased by 364.4 in the experimental group, but increased by 19.2 in the control group. As a result of the test of the changes in the outstanding pores, it is found there is a statistically significant difference between the two groups with t = 4.572 and p < 0.001. The large pore

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Table 8 The comparison in the group KAGS N(%)

KAGS	Myrtle			Control			t	р		
	Week	k		Week	Week	Week				
	0	3	6	0	3	6				
0	0(0)	1(10)	3(30)	0(0)	0(0)	0(0)	3.151	0.006**		
1	5(50)	7(10)	7(30)	6(60)	5(50)	6(60)				
2	3(30)	1(70)	0(0)	2(20)	4(40)	3(30)				
3	1(10)	1(10)	1(10)	2(20)	1(10)	1(10)				
4	1(10)	0(10)	0(0)	0(0)	0(0)	0(0)				
$M \pm SD$	1.8 ± 1.0		$0.9 \pm 0.9**$	1.6 ± 0.8		1.5 ± 0.7				

p < 0.01 is present as **

index decreased by 4.3 in the experimental group, but increased by 1.1 in the control group. As a result of test of the changes in the large pores, it is found there is a statistically significant difference between the two groups with t=3.16 and p=0.005. The blackhead index decreased by 362.0 in the experimental group, but increased by 49.6 in the control group. As a result of test of the changes in the blackhead, it is found there is a statistically significant difference between the two groups with t=5.130 and p<0.001 (Table 9).

Changes in erythema index

The erythema index decreased by 27.5 in the experimental group, but increased by 7.8 in the control group. However, as a result of test of the changes, it is found there is no statistically significant difference between the two groups with t = 1.833 and p = 0.083 (Table 10).

Changes in sebum index on surface of skin

The sebum index decreased by 2.4 in the experimental group, but increased by 0.6 in the control group. As a result of test of the changes, it is found there is a

statistically significant difference between the two groups with t = 2.313 and p = 0.033 (Table 11).

Changes in desquamation index on surface of skin

The desquamation index decreased by 98.9 in the experimental group, but increased by 35.6 in the control group. As a result of test of the changes, it is found there is a statistically significant difference between the two groups with t = 4.34 and p < 0.001 (Table 12). There occurred something noteworthy in the desquamation index in week 3. In view of the statistics in week 3 of the experimental group, it is found there is almost no change in the average and the standard deviation compared to those figures in week 0, but there is a great difference in the desquamation index between subjects in the experimental group. The subjects in the experimental group were divided into two groups: one with an increased desquamation index in week 3, which is an early stage of the test, and a remarkably decreased desquamation index in week 6; and the other with a decreased desguamation index both in week 3 and week 6. However, the division in week 3 disappeared in week 6, with all

Table 9 The comparison in weekly average of outstanding pores, large pores, and blackheads

		Myrtle M(± SD)	Control M(± SD)	t	p
Outstanding pores	0 week	1271.9(± 677.3)	1127.7(± 905.9)	4.572	0.000**
	3 week	1080.8(± 586.7)	1132.5(± 799.9)		
	6 week	907.5(± 484.6)	1146.9(± 853.8)		
	0–6 gap	- 364.4(± 232.2)	+ 19.2(± 128.4)		
Large pores	0 week	38.8(± 46.4)	30.9(± 54)	3.160	0.005**
	3 week	35.1(± 44.5)	31.2(± 53.5)		
	6 week	34.5(± 43.4)	31.9(± 54)		
	0–6 gap	$-4.3(\pm 4.9)$	$+ 1.1(\pm 2.3)$		
Blackheads	0 week	649.2(± 468.2)	569.5(± 630.1)	5.130	0.000**
	3 week	508.5(± 342.4)	629.1(± 662.9)		
	6 week	287.2(± 229.8)	619.1(± 647.1)		
	0–6 gap	$-362.0(\pm 244.6)$	+ 49.6(± 67.6)		

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Table 10 The comparison in group of erythema

	Myrtle M(± SD)	Control M(± SD)	t	р
Week 0	392.5(± 62.5)	378.3(± 47.9)	1.833	0.083
Week 3	379.5(± 57.9)	387.5(± 68.3)		
Week 6	365(± 48.4)	386(± 68.2)		
Gap (week 0–6)	$-27.5(\pm 34.3)$	$+7.8(\pm 50.2)$		

subjects in the experimental group having decreased dead skin cells in week 6.

Changes in microorganism index on surface of skin

The microorganism index decreased by 3334.5 in the experimental group and decreased by 335.2 in the control group. As a result of the test of the changes in the microorganism index, it is found there is a statistically significant difference between the two groups with t = 2.897 and p = 0.009 (Table 13).

Discussion

This study applied myrtle essential oil, which has no irritation, no toxicity, and no sensitivity among natural materials (Lawless 1995), to acne skin to see the effects. As a result of the tests, all of the acne grades, the erythema index, the pore index, the desquamation index, and the microorganism index showed an improvement, with a statistically significant result. An improved acne grade, according to the KAGS criteria, refers to a reduced number of inflammatory acne. In the process of this study's tests, there are two major factors to affect changes in acne grades: the act of pressing out acne; and the application of myrtle substances. However, the act of pressing out acne was equally done to the control group, leading to no great change in the acne grades. Therefore, it can be learned that the myrtle substances have had a direct impact on improving the acne grades, while the act of pressing out acne with no effective substances applied has been found to bring no great improvement.

It can be seen that, by analyzing an improvement of the acne grades based on the mechanism of generation of acne, an improved acne grade results from a combination of removal of dead skin cells and sebum,

Table 11 The comparison in group of sebum

	Myrtle M(± SD)	Control M(± SD)	t	р
Week 0	7.6(± 2.7)	8.7(± 5.4)	2.313	0.033*
Week 3	$6.7(\pm 2.4)$	9.5(± 5.4)		
Week 6	5.2(± 2.7)	9.2(± 4.3)		
Gap (week 0–6)	$-2.4(\pm 2.7)$	$+ 0.6(\pm 2.9)$		

p < 0.05 is present as *

Table 12 The comparison in group of desquamation

	Myrtle M(± SD)	Control M(± SD)	t	р
Week 0	245.2(± 95.2)	232.5(± 101.5)	4.340	0.000**
Week 3	241.9(± 97.8)	252.4(± 97.5)		
Week 6	146.3(± 75.4)	268.1(± 96.1)		
Gap (week 0-6)	- 98.9(± 91.3)	+ 35.6(± 35.5)		

p < 0.01 is present as **

antibacterial activity, anti-inflammatory activity, and soothing activity (Del Rosso et al. 2016). An improved microorganism index can be viewed as a result from anti-bacterial activity by oxide-based 1,8-cineole and mono terpene-based α-pinene, and limonene. It has been reported that 1,8-cineole has anti-inflammatory and antibacterial effects by suppressing the pathway of leukotriene B4 and prostaglandin E2 in blood mononuclear cells (Bowles 2000). Merghni et al. have cited that there had been a study reporting that 1,8-cineole has a considerable level of anti-microorganism activity gram-positive species and gram-negative ones (Merghni et al. 2018). This study also confirmed the antibacterial effects of 1,8-cineole, which is a component of myrtle. In addition, α-pinene and limonene interact with the receptor system in energy metabolism in living bodies to suppress oxygen intake and ATP synthesis, also inhibiting enzymatic actions (Hale et al. 2015). Accordingly, an improved microorganism index may be regarded as a result from antibacterial effects of 1,8-cineole, α-pinene, and limonene. This study also found out that an improved sebum index results from a lipophagy activity of 1,8-cineole, α-pinene, and limonene (Battaglia 1997). A lipophagy dissolves intercellular lipid in dead skin cells, inducing the dead skin cells to fall off. Especially, 1,8-cineole, an oxide substance, can present a synergy effect in removing dead skin cells by dissolving keratin protein (Merghni et al. 2018; Battaglia 1997). With regard to the desquamation index changes, there occurred something noteworthy in week 3. In view of the statistics of the experimental group in week 3, there shows almost no change in the average and the standard deviation from those in week 0. However, there showed great differences in the desquamation index between the

Table 13 The comparison in group of skin microorganism

		<u>'</u>		
	Myrtle M(± SD)	Control M(± SD)	t	р
Week 0	8343.9(± 3486.6)	7883.3(± 2192.8)	2.897	0.009**
Week 3	6436.2(± 2710.4)	7555.7(± 2252.9)		
Week 6	5009.4(± 1863.3)	7548.1(± 2426)		
Gap (week 0-6)	- 3334.5(± 2784.6)	- 335.2(± 1722.8)		

p < 0.01 is present as **

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subjects in the experimental group. The subjects in the group were divided into two groups: one with an increased desquamation index in week 3, which is an early stage of the tests, and a remarkably decreased desquamation index in week 6; and the other with a decreased desquamation index both in week 3 and week 6. However, the division in week 3 disappeared in week 6, and all subjects in the experimental group experienced a decrease in dead skin cells. This is considered to show that there is a difference in the desquamation index in the early stage of the tests since the desquamation cycles and the condition of dead skin cells vary from person to person (Kim and Choi 2018). By a morphological observation of the skin when the dead skin cells are removed, edema occurs in the basal layer and the upper layer of the thick skin because of an increase in cell activity including an appearance of vimentin thin filament in fibroblast in the thick skin or vascular endothelial cells (Sandoval et al. 2014). An increased edema and activity in the basal layer and the thick skin may be considered a change in reproduction of the skin according to a healing process for a cut. Accordingly, an increased skin elasticity and tightened pores, which occur after dead skin cells are removed, may be regarded as a result from an increase in activity in the basal layer and the upper layer of the thick skin. The results of the tests of this study also confirmed an improved pore index due to a better desquamation index. In addition, it can be learned that the skin has not been made more sensitive by removing dead skin cells from the results of the erythema index. In view of the results of the erythema index, it can be reconfirmed that myrtle not only has effects for removal of dead skin cells and sebum and convergence but also is a safe substance with skin-soothing effects without making the skin more sensitive. It can be learned that, since myrtle simultaneously contains ester-based myrtenyl acetate, which soothes the skin and recovers the balance in the skin, oxide-based 1,8-cineole with effects for desquamation, convergence, and antibacterial and anti-inflammatory activity, and mono terpene-based α-pinene and limonene, it presents excellent effects for improving acne skin by synergy effects between activity substances and soothing substances, also serving as a safe substance with low irritation.

Conclusions

This study applied myrtle essential oil, which contains ester, oxide, and mono terpene, to acne skin to see changes in the skin. This study made foam cleanser, toner, emulsion, and cream pack with myrtle essential oil added to apply them to the facial skin of the subjects. To precisely check the effects of the myrtle substances, there was only a single difference of the addition or no

addition of myrtle between the experimental group and the control group, leaving all other conditions equal.

First, the average acne grade significantly decreased from grade 1.8 to grade 0.9 in the experimental group; but, it showed only a statistically insignificant decrease in the control group from grade 1.6 to grade 1.5.

Second, as a result of analysis of pore index, the outstanding pore index decreased by 364.4 in the experimental group, but increased by 19.2 in the control group; the large pore index decreased by 4.3 in the experimental group, but increased by 1.1 in the control group; and, the blackhead index decreased by 362.0 in the experimental group, but increased by 49.6 in the control group. In summary, all of the three pore indices showed a statistically significant decrease in the experimental group.

Third, the erythema index decreased by 27.5 in the experimental group, but increased by 7.8 in the control group. However, the changes in the erythema index did not show a statistically significant difference between the two groups.

Fourth, the sebum index decreased by 2.4 in the experimental group, but increased by 0.6 in the control group. In addition, the changes in the sebum index showed a statistically significant difference between the two groups.

Fifth, the desquamation index decreased by 98.9 in the experimental group, but increased by 35.6 in the control group. The changes in the desquamation index showed a statistically significant difference between the two groups.

Sixth, the microorganism index decreased by 3334.5 in the experimental group, but decreased by 335.2 in the control group. The changes in the microorganism index showed a statistically significant difference between the two groups.

This study has clinically proved that myrtle essential oil has effects for convergence, reduction of erythema, removal of sebum and dead skin cells, and antibacterial activity in the skin of Korean women. Especially, it is confirmed that myrtle essential oil is a safe effective substance for treating acne with skin-soothing effects from the results of reduced erythema.

Abbreviations

DI: Desquamation index; EI: Erythema index; KAGS: Korean acne grading system

Acknowledgements

The authors thank all the study subjects who participated in this work.

Funding

Not applicable.

Availability of data and materials

Not applicable

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Authors' contributions

KK and SA designed the study. HHJ, SNL, and YSK did all of the research such as the experiments, data collection, and statistical analysis. KK and SA wrote the draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The subjects were informed about all information with regard to the test and took part in the test after signing a written consent according to their will.

All clinical tests for this study have undergone the deliberation of the Institutional Review Boards before starting the tests (NO. 1-70005239-AB-N-01-2018-KIDS-AHA043-HR-01).

Consent for publication

Not applicable.

Competing interests

Sungkwan An is the co-Editor-in-Chief. There was no funding for this article. The authors declare that they have no competing interests.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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Received: 22 August 2018 Accepted: 14 November 2018 Published online: 10 December 2018

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