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Function of Blending Essential Oil in the Development of Anti-Dandruff Products

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Abstract

Purpose*: In this paper, we show our blending ratio of 10 types of Essential Oils that survives beneficial bacteria and kills harmful bacteria in the scalp, and we investigate the possibility of application of our blending ratio to the development of anti-dandruff products and the possibility of being used as a raw material for clinical beauty and customized cosmetics.*

Methods*: The scalp microorganisms used in our study were M. furfur, S. epidermidis, E. coli, and P. nitroreducens. There are a total of 10 Essential Oils such as True Lavender, Lime, Roman chamomile, Rosemary camphor, Cedarwood, Geranium, Clove, Tea tree, Palmalosa, and Peppermint. The antibacterial test of the blended Essential Oil was carried out according to the test method of the standardized evaluation methodology of "Food and Food Additives Code". Since M. furfur is related to the growth of sebum in the scalp, in this study we used the fnLNB and the fnLNA with 20 ㎖ of whole fat cow milk added.*

Results*: The blending ratio of EO, which inhibits dandruff-causing bacteria such as M. furfur, S. epidermidis, E. coli, and does not inhibit P. nitroreducens showing dominant growth in a healthy scalp, was B8(Clove 0.2%, Roman chamomile 0.5%, Tea tree 0.3%), B9(Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.3%), B10(Clove 0.1%, Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.2%).*

Conclusion*: It is thought that the blending ratio of BEO obtained as a result of this study can provide a basis for use as an alternative to antibiotics in developing anti-dandruff drugs and emerge as a new alternative to solve scalp microbial imbalance. In order for EO to be used as a useful raw material for anti-dandruff preparation, researches on 1) Standardization (the effects of products differ according to the types, regions, climate, extraction methods, etc.), 2) Antimicrobial effects, 3) Safety, etc., must be established.*

Key words*: Blending Essential Oil, Antibacterial Effect, Hair Loss, Scalp Microbial Environment, Substitute for Antibiotics.*

1. Introduction

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Dandruff appears visually as white or yellow pieces and negatively affects a person's appearance. Dandruff often causes itching, tingling, a feeling of pressure, pain and erythema of the scalp, causing discomfort to the patient [1]. The prevalence of dandruff, which does not involve erythema, indicates that adults have a incidence of nearly 50%, and it has been suggested that the causes of dandruff vary depending on individual sensitivity, sebum secretion, and *Malassezia. spp*. [2, 3]. In addition, it has been reported that dandruff is caused by the predominance of *Malassezia.spp.* in the scalp, and it has been reported that the colony of microorganisms is unbalanced [3-5].

Essential Oil (EO) has the effect of inhibiting the growth of fungi and bacteria that cause dandruff, but it does not inhibit dandruff in a wide range compared to antibiotics such as Ketoconazole (KET) [6, 7]. However, compared to KET, EO has secured a safety to resolve side effects, and it is expected that it will be able to resolve concerns about recurrence due to antibiotic resistance [8, 9].

In previous studies, we proposed a minimum inhibitory concentration blending ratio and volatile oil that can resolve the imbalance of microbial clusters present in dandruff scalps, according to the results of inhibiting harmful bacteria without inhibiting beneficial bacteria by blending EO [10, 11],.

In this study, using the results of previous studies, we examined whether it can be used as an anti-dandruff material development, clinical beauty, and raw materials for customized cosmetics, by finding and applying various blending ratios so that individuals can choose their own preferred fragrance.

2. Experiment

2.1 Materials

2.1.1 Test strains. As shown in Table.1,

Table 1. Microorganism species used in the study

Strain	No. of strain	Media
M. furfur	KCTC 7743	fmLNB, fmLNA)
S. epidermidis	KCTC 14990	TSB, TSA
E. coli	ATCC 25922	TSB, TSA
P. nitroreducens	KCTC 12325	TSB, TSA

the microbiota we used in this study were dandruff-causing microorganisms, such as *Malassezia furfur* (*M. furfur*, KCTC 7743), *Staphylococcus epidermidis* (*S. epidermidis*, KCTC 14990), *Escherichia. Coli* (*E. coli*, ATCC 25922) and *Pseudomonas nitroreducens* (*P. nitroreducens*, KCTC 12325) which were predominant in healthy scalps [12-14].

2.1.2 Media. In this study, for *M. furfur* we used a liquid medium in which Whole fat cow milk 20ml(Seoul milk, Korea) is added to mLNB(Leeming & Notman Broth modified, KisanBio, Korea), and a solid medium in which Whole fat cow milk 20ml(Seoul milk, Korea) and 15 g of Agar (Duksan, Korea) added to mLNB (fmLNA); and for *S. epidermidis*, *E. coli, P. nitroreducens*, we used TSB(Tryptic Soy Broth, Difco, USA)와 TSA(Tryptic Soy Agar, Difco, USA).

2.1.3 EO. As shown in Table 2,

Table 2. EOs used in the study

EO	Botanical name	brand name
True Lavender	*Lavendula angustifolia*	ameo
Lime	*Citrus auranifoli*	ameo
Roman chamomile	*Anthemis nobilis*	ameo
Rosemary camphor	*Rosmarins officinalis*	FLORIHANA
Cedarwood	*Cedrus atlantica*	FLORIHANA
Geranium	*Pelargonium graveolens*	La Sélection
Clove	*Eugenia caryophyllus*	FLORIHANA
Tea tree	*Melaleuca alternifolia*	ameo
Palmarosa	*Cymbopogon martini*	La Sélection
Peppermint	*Mentha piperita*	ameo

there are a total of 10 types of EOs used in this study, such as True Lavender, Lime, Roman chamomile, Rosemary camphor, Cedarwood, Geranium, Clove, Teatree, Palmalosa, and Peppermint.

2.2 Methods

2.2.1 Preparation of Test Bacteria. The test strains such as *E. coli, S. epidermidis* and *P. nitroreducens* were subcultured 3 times for 24 hours at 36°C by streaking on TSA in order to confirm the homogeneity of the bacteria and used in the experiment. We used a technique of streaking on the fmLNA medium for *M. furfur*, and subcultured three times at 30°C for 48 hours to confirm the homogeneity. In order to use in our experiments, we cultured *E. coli*, *S. epidermidis*, and *P. nitroreducens* in TSB at 36°C for 24 hours, and cultured M. furfur at 30°C in fmLNAB for 48 hours.

2.2.2 Antibacterial test of Single Essential Oil (SEO). The antibacterial test of SEO was carried out according to the test method of the standardized evaluation methodology of Food and Food Additives Code. In the case of *E. coli, S. epidermidis*, and *P. nitrideducens*, SEO was inoculated into the TSB medium at concentrations of 2%, 1%, 0.5%, and 0.1%, respectively.

In addition, Tween 80 was added and vortexed and the 10μl of prepared test strain solution was inoculated, and vortexed at 3000 rpm for 2 minutes those were cultured at 36°C for 24 hours. We measured the number of bacteria by incubating them at 36°C for 24 hours by using a 10-fold dilution method for spreading in fmLNA plate.

In addition, for *M. furfur*. SEO was inoculated into fmLNB medium at concentrations of 2%, 1%, 0.5%, and 0.1%. In addition, Tween 80 was added and vortexed and the 100μl of prepared test strain solution was inoculated and vortexed, and those were cultured 30°C for 48 hours. We measured the number of bacteria by incubating them at 30°C for 48 hours by using a 10-fold dilution method for spreading in fmLNA plate.

2.2.3 Antibacterial test of BEO

The antibacterial test of BEO was carried out according to the test method of the standardized evaluation methodology of "Food and Food Additives Code" [**15**]. According to the results obtained in the antibacterial test of SEO, BEO was injected as shown in Table 3.

Table 3. Blending ratio of BEO used in this study

Name of Essential Oil	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B 10
Cedarwood						0.10%	0.20%			
Clove	0.50%	0.50%	0.25%	0.25%	0.10%			0.20%		0.20%
Geranium	1.00%	1.00%	0.50%	0.25%	0.10%		0.10%		0.10%	0.10%
Lime						0.10%	0.10%			
Palmarosa	1.00%	1.00%	0.50%	0.25%	0.10%				0.10%	0.10%
Peppermint						0.10%				
Roman chamomile	2.00%	1.00%	1.50%	0.75%	0.5%	0.20%		0.50%	0.50%	0.50%
Rosemary camphor										
Tea tree	1.00%	1.00%	0.50%	0.25%	0.10%		0.20%	0.30%	0.30%	0.20%
True lavender	1.00%		0.50%	0.25%	0.10%	0.20%	0.10%			
Total amount	6.50%	4.50%	3.75%	2.00%	1.00%	0.70%	0.70%	1.00%	1.00%	1.00%

For *E. coli, S. epidermidis*, and *P. nitroreducens*, Blending1(B1)-Blending10(B10) was injected into TSB, Tween 80 was added and vortexed and the 10μl of prepared test strain solution was inoculated and vortexed at 3000 rpm for 2 minutes those were cultured at 36°C for 24 hours. We measured the number of bacteria by incubating them at 36°C for 24 hours by using a 10-fold dilution method for spreading in TSA plate.

Further, in the case of *M. furfur*, B1-B10 were inoculated into the fmLNB plate; and in addition, Tween 80 was added and Vortexed and the 100μl of prepared test strain solution was inoculated and Vortexed again, and those were cultured at 30°C for 48 hours. We measured the number of bacteria by incubating them at 30°C for 48 hours by using a 10-fold dilution method for spreading in fmLNA Plate.

3. Results

3.1 Antibacterial effect of BEO on *E. coli*

The antimicrobial effect of BEO on *E. coli* was as shown in Figure. 1.

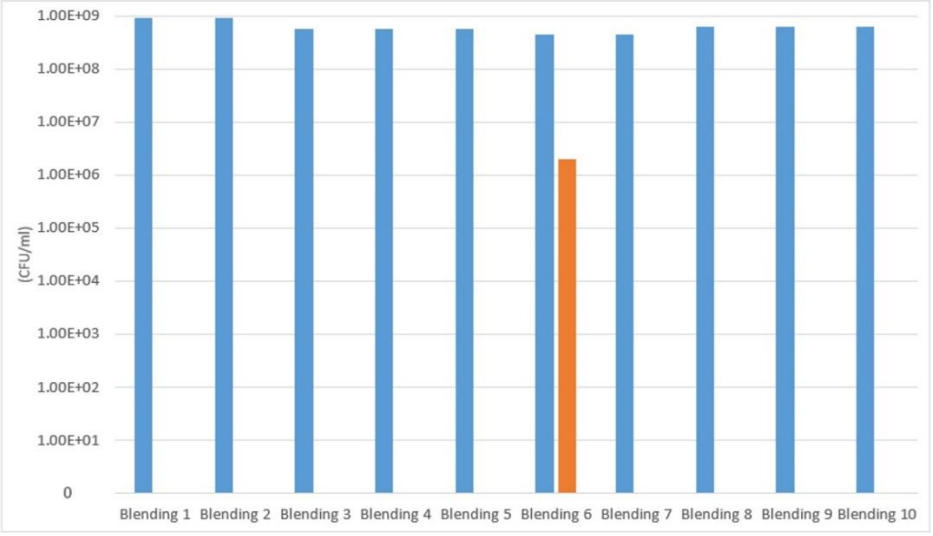

Figure 1. Antibacterial effect of BEO on *E. coli*

represents a control group in which tween80 was mixed well with TSB without essential oil and inoculated with *E. coli*.

represents a blending essential oil group and after adding blending essential oil in TSB, mixing with tween80 and inoculating *E. coli*.

In the case of *E. coli*, the antibacterial effect of BEO showed that it completely killed *E. coli* in all blendings except B6.

B6 is a combination of Cedarwood 0.1%, Lime 0.1%, Peppermint 0.1%, Roman chamomile 0.2%, and True lavender 0.2%, and the total amount of EO is 0.7%.

3.2 Antibacterial effect of BEO on *S. epidermidis*

The antimicrobial effect of BEO on *S. epidermidis* was as shown in Figure 2.

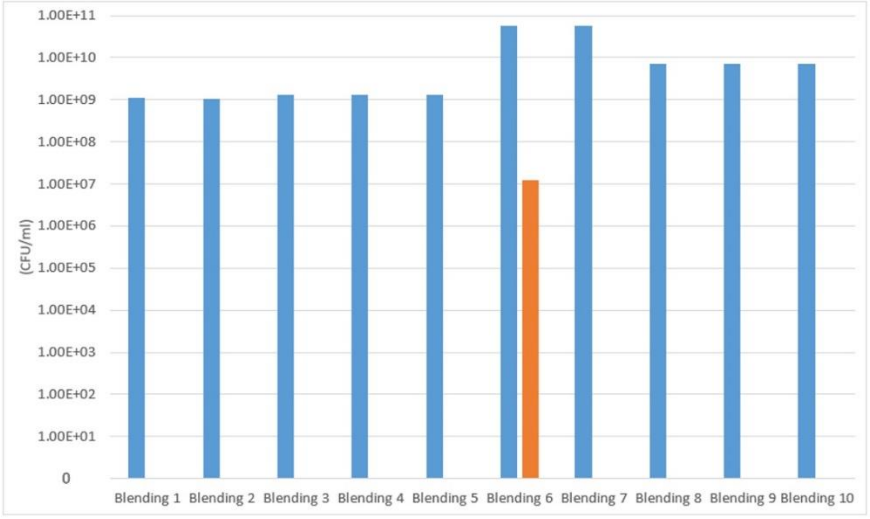

Figure 2. Antibacterial effect of BEO on *S. epidermidis*

represents a control group in which tween80 was mixed well with TSB without essential oil and inoculated with *S. epidermidis*

represents a blending essential oil group and after adding blending essential oil in TSB, mixing with tween80 and inoculating *S. epidermidis*

In the case of *S. epidermidis,* the antibacterial effect of BEO showed that it completely killed *S. epidermidis* in all blendings except B6.

B6 had a similar antibacterial effect on *S. epidermidis* as the antibacterial effect on *E. coli* but showed an antibacterial effect that killed 10^1 more *S. epidermidis* than *E. coli*.

3.3 Antibacterial effect of BEO on *P. nitroreducens*

The antimicrobial effect of BEO on *P. nitroreducens* was shown in Figure 3.

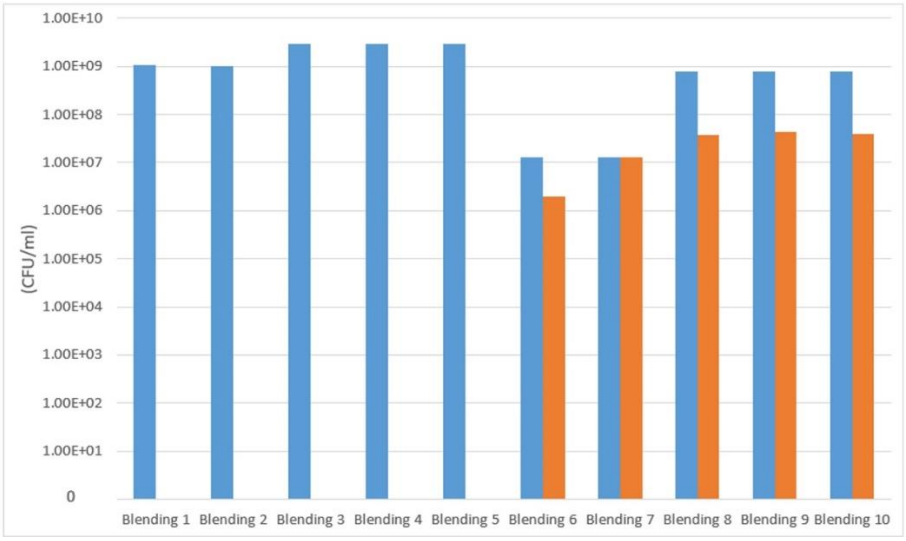

Figure 3. Antibacterial effect of BEO on *P. nitroreducens*

represents a control group in which tween80 was mixed well with TSB without essential oil and inoculated with *P. nitroreducens*

represents a blending essential oil group and after adding blending essential oil in TSB, mixing with tween80 and inoculating *P. nitroreducens*

In the case the antibacterial effect of BEO on *P. nitroreducens*, a blending of B1-B5 showed that it completely killed *P. nitroreducens* in all blendings except B6, but a blending of B6-B10 showed that it did not completely killed *P. nitroreducens*. A blending B1-B5 the one that is made with Clove, Geranium, Palmarosa, Roman chamomile, Tea tree and True lavender (with B2 excluding a True lavender).

3.4 Antibacterial effect of BEO on *M. furfur*

The antimicrobial effect of BEO on *M. furfur* was shown in Figure 4.

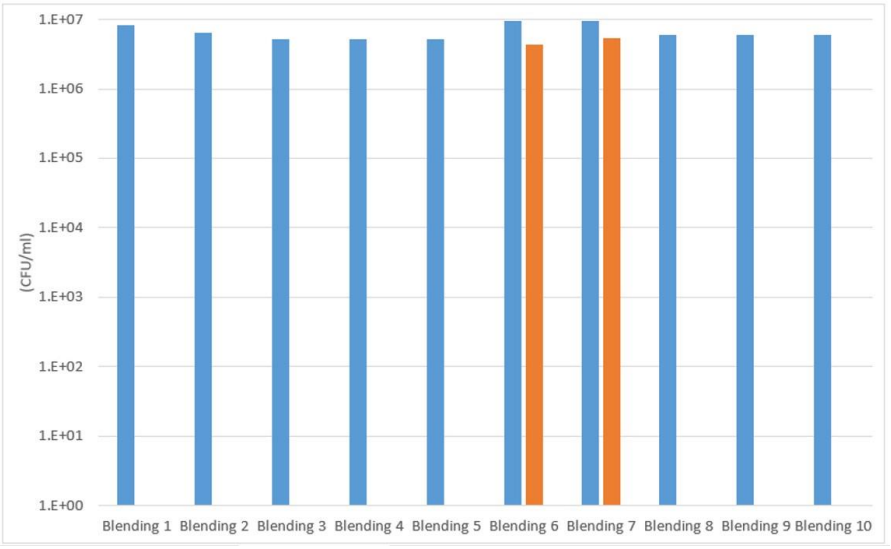

Figure 4. Antibacterial effect of BEO on *M. furfur*

represents a control group in which tween80 was mixed well with TSB without essential oil and inoculated with *M. furfur*
represents a blending essential oil group and after adding blending essential oil in TSB, mixing with tween80 and inoculating *M. furfur*

In the case of *M. furfur*, the antibacterial effect of BEO showed that it completely killed *M. furfur* in all blendings except B6 and B7; and in the case of B6 and B7, a blending B6 is a combination of Roman chamomile added concentration of 0.2% and a blending B7 is a combination without Roman chamomile added at all. According to this result, antibacterial effect was shown when Roman chamomile was 0.5 or more against M. furfur.

3.5 Blending ratio of EO that inhibits *M. furfur, S. epidermidis*, and *E. coli* but does not inhibit *P. nitroreducens*

Table 4 shows the ratio of EO that inhibits dandruff-causing bacteria, *M. furfur*, *S. epidermidis*, and *E. coli*, but does not inhibit *P. nitroreducens*, which shows dominant growth on a healthy scalp.

Table 4. Antibacterial effect of BEO that inhibits *M. furfur, S. epidermidis,* and *E. coli* but does not inhibit *P. nitroreducens*

Name of Essential Oil	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B10
M. furfur	-	-	-	-	-	+	+	-	-	-
S. epidermidis	-	-	-	-	-	+	-	-	-	-
E. coli	-	-	-	-	-	+	-	-	-	-
P. nitroreducens	-	-	-	-	-	+	+	+	+	+

The blending ratios of EO, which inhibits the dandruff-causing bacteria, such as *M. furfur, S. epidermidis*, and *E. coli*, and does not inhibit *P. nitroreducens*, which shows dominant growth in healthy scalp, were B8 (Clove 0.2%, Roman chamomile 0.5%, Tea tree 0.3%), B9 (Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.3%), and B10 (Clove 0.1%, Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.2%).

B1-B5 showed an antibacterial effect that completely killed all of *M. furfur, S. epidermidis, E. coli*, and *P. nitroreducens*, B6 showed no antibacterial effect on *M. furfur, S. epidermidis, E. coli*, and *P. nitroreducens*, and B7 showed an antibacterial effect that did not inhibit *M. furfur* and *P. nitroreducens*, but inhibited *S. epidermidis and E. coli.*

4. Discussion

EO, a secondary metabolite of various plants, is defined as a volatile substance obtained by physical methods from the secondary metabolites of various aromatic plants, produced by one single plant type and plant species. EO is used as a raw material in the food and fragrance industry, perfume industry, pharmaceutical industry, chemical manufacturing industry, and cosmetics industry. In order for EO to be used as a useful raw material for anti-dandruff preparation, researches on 1) Standardization (the effects of products differ according to the types, regions, climate, extraction methods, etc.), 2) Antimicrobial effects, 3) Safety, etc., must be established.

In this study, we tested whether EO has an antibacterial effect on dandruff-causing bacteria (such as *M. furfur, S. epidermidis, E. coli*) and whether it can resolve the imbalance of scalp microorganisms that cause dandruff development. By blending True Lavender, Lime, Roman chamomile, Rosemary camphor, Cedarwood, Geranium , Clove, Tea tree, Palmalosa, Peppermint, etc., which are known to have antibacterial effects to resolve the imbalance of scalp microorganisms, we tried to find the blending ratio of BEO that does inhibit dandruff-induced *M. furfur* and does not *inhibit P. nitroreducens*, which is dominant in healthy scalp [7, 16-21].

In the case of antibacterial effects on E. coli, previous studies have shown that Grapefruit seed extract, Eucalyptus oil, Tea tree oil mixture solution, Grapefruit seed extract (GSE), Lemongrass and Thyme mixture solution, GSE, Cinnamon, Ginger mixture solution, GSE, Eucalypt, Tea tree mixture solution showed antibacterial effects against E. coli [10-11]. However, in this study, we showed that the blending ratios B1-B5 and B7-B10 in Table 3 showed an antibacterial effect that completely kill *E. coli*. But, B6 had no antibacterial effect. The difference other Blendings, except for B6 and B7, have is that Clove and Tea tree were not included in the combination. The total amount of B6 and B7 was the same, but the difference was that B6 contained Peppermint and Roman chamomile, and B7 contained Geranium and Tea tree.

In the case of antimicrobial effects on *S. epidermidis*, previous studies reported that the mixture of lavender, clary sage, and ylang ylang did show an inhibitory effect on *S. epidermidis*, and reported that the mixture of petigrain, clary sage, and jasmine did not showed an inhibitory effect on *S. epidermidis* [22]. However, in this study, although there is a difference in the number of bacteria to be inhibited, B1-B5 and B7-B10, like *E. coli*, showed an antibacterial effect that completely kills *S. epidermidis*. B6 had no antibacterial effect. The difference other Blendings, except for B6 and B7, have is that Clove and Tea tree were not included in the combination. The total amount (0.7%) of B6 and B7 was the same, but the difference was that B6 contained Peppermint and Roman chamomile, and B7 contained Geranium and Tea tree. It can be said that the

antibacterial effect on *E. coli* and *S. epidermidis* is determined by the presence or absence of Clove, Geranium, and Tea tree.

As for the antibacterial effect on M. furfur, previous studies reported that the mixed solution (of Cinnamon and Kapur tulsi) and the mixed solution (of Cinnamon, Kapur tulsi, and Cajeput) had no inhibitory effect on *M. furfur* [6]. However, in this study, we showed the antibacterial effect of completely killing *M. furfur* in other blendings except B6 and B7. The differences between B6, B7, and other blendings are as follows. B6 contains 0.2% Roman chamomile, B7 does not contain Roman chamomile, and the remaining Blendings contain more than 0.5% Roman chamomile. Therefore, it can be said that the antibacterial effect on *M. furfur* depends on the concentration and presence of Roman chamomile.

P. nitroreducens was not inhibited in B6-B10 but completely killed in B1-B5.

The blending ratio of EO, which inhibits dandruff-causing bacteria such as *M. furfur, S. epidermidis, E. coli,* and does not inhibit *P. nitroreducens* showing dominant growth in a healthy scalp, was B8(Clove 0.2%, Roman chamomile 0.5%, Tea tree 0.3%), B9(Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.3%), B10(Clove 0.1%, Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.2%).

According to the results, BEO has solved the side effects and antibiotic resistance problems such as ketoconazole, clotrimazole, and miconazole, which are antifungal agents used in dandruff treatment; and provided the basis for solving scalp microbial imbalance. To confirm whether BEO can be used as an antibiotic substitute, it is necessary to greatly improve the EO-containing product market in the future, by conducting various antibacterial tests of more essential oil, development of various blending ratios of EO that can solve scalp microbial imbalance, and safety tests.

5. Conclusion

The blending ratio of EO, which inhibits dandruff-causing bacteria such as *M. furfur, S. epidermidis, E. coli,* and does not inhibit *P. nitroreducens* showing dominant growth in a healthy scalp, was B8(Clove 0.2%, Roman chamomile 0.5%, Tea tree 0.3%), B9(Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.3%), B10(Clove 0.1%, Geranium 0.1%, Palmarosa 0.1%, Roman chamomile 0.5%, Tea tree 0.2%), and BEO B6 and B7, which have a total amount of 0.7%, have a smaller antibacterial effect than B1-B5 and B8-B10, which are 1% or more. Geranium, Palmaros, Roman chamomile, and tea tree, the total mixing amount is 1.0%, and Roman chamomile was found to be the best mixing ratio when 0.5% of them. It is thought that the blending ratio of BEO obtained as a result of this study can provide a basis for use as an alternative to antibiotics in developing anti-dandruff drugs and emerge as a new alternative to solve scalp microbial imbalance.

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Conflict of interest: The authors declare that there are no conflicts of interest

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