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Antimicrobial and hand hygiene effects of Tea Tree Essential Oil disinfectant: A randomised control trial

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Abstract

Background: Hand hygiene is paramount in preventing healthcare-associated infections in medical environments and the spread of infectious diseases in non-medical environments.

Aims: This study used a randomised controlled trial to investigate the effects of a tea tree (Melaleuca alternifolia) oil disinfectant on hand disinfection and skin condition.

Methods: A tea tree oil group received 5 mL of 10% tea tree oil disinfectant mixed in a ratio of 2:2:1:15 of Melaleuca alternifolia oil, solubiliser, glycerin and sterile distilled water. Data collection took place between April 9 and April 13, 2018. The subjects were 112 healthy adults. An alcohol group received 2 mL of a gel-type hand sanitiser comprising 83% ethanol used without water; a benzalkonium chloride group received 0.8 mL of a foam-type hand sanitiser containing benzalkonium chloride used without water and a control group received no treatment. Subjective skin condition, transepidermal water loss and adenosine triphosphate were assessed, and a microbial culture test was performed following treatment.

Results: The general characteristics and the pretreatment dependent variables did not differ significantly by group. Posttreatment adenosine triphosphate $\log_{10}$ values significantly differed across all four groups ($F = 3.23$, $P = .025$). Similarly, posttreatment bacterial density $\log_{10}$ values differed significantly across the tea tree oil, alcohol, benzalkonium chloride and control groups ($F = 91.71$, $P < .001$).

Conclusion: The study confirmed that tea tree oil disinfectant is effective for hand disinfection. Accordingly, tea tree oil disinfectants may be introduced to nursing practice as a new hand hygiene product to prevent and reduce healthcare-associated infections.

1 | INTRODUCTION

Hand hygiene is paramount in preventing healthcare-associated infections in medical environments. It is also important for preventing the spread of infectious diseases in non-medical environments.[1] It is a key interventional method for effectively stopping the spread of pathogens through person-to-person contact and person-to-surface contact.[2] Furthermore, hand hygiene can alleviate risks and reduce the incidence of infection in working environments.[3,4]

Hand hygiene is a simple and effective method for preventing healthcare-associated infection.[5] Although hand hygiene awareness is increasing, actual hand hygiene performance rates in medical institutions vary from 5% to 89%.[6] Obstacles to hand hygiene include a lack of time, a lack of peer and superior role models,[7] inadequate hand hygiene facilities, a lack of accurate knowledge and willingness regarding hand hygiene[8] and skin irritation associated with hand hygiene.[9] Skin irritation occurs following frequent and repeated use of hand hygiene products, such as soap and detergent, which can also cause chronic

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contact dermatitis.[10,11] Detergents can cause skin damage through denaturing stratum cornea proteins, intercellular lipid changes, decreased cohesion of keratinocytes and decreased water-binding capacity of the stratum corneum.[12] Alcohol induces skin dryness and irritation[13] and further irritates skin already damaged by detergents, making it more sensitive.[14] Moreover, skin damage caused by hand hygiene alters the distribution of normal flora, leading to increased levels of staphylococcus and gram-negative bacteria.[15,16]

Alcohol-based hand rubs that obviate the need for water were introduced to improve hand hygiene performance rates[17]; however, this led to an increase in the prevalence of hand dermatitis.[18] In addition, biocide injuries caused by the misuse or abuse of chemicals have recently emerged as a social issue,[19] causing anxiety about the potential toxicity from frequent exposure to disinfectants.

As hand sanitisers made of chemical disinfectants caused many types of skin troubles, researchers have been conducting further studies on hand hygiene formulations.[20] One study that assessed hand hygiene formulations using tea tree essential oil demonstrated antimicrobial activity with no skin irritation or dryness despite repeated use.[21] Aromatherapy essential oils can offer antimicrobial, wound healing, cell regeneration and immune enhancement effects depending on the composition and chemical properties, and they can be used safely as they have few adverse effects.[22] Tea tree oil has shown broad-spectrum antimicrobial activity against bacteria, viruses and fungi.[23]

The antimicrobial effects of hand sanitisers can be examined in various ways by in vitro tests. However, the actual application of disinfectant to the skin can identify differences in effects related to a disinfectant's characteristics and skin conditions based on various organic materials, microorganisms and contaminants found on the skin. Therefore, in vivo tests involving direct skin use have greater sensitivity and are more effective.[24] To test microbial reduction effects, there are various methods for measuring the number of colonies on a hand, including swabbing, the fingertip method and the glove juice sample method. Among these, the glove juice sample method is a highly sensitive method that accurately estimates the number of microorganisms colonised on a hand.[24,25]

Accordingly, the present study applied the FDA-Tentative Final Monograph for Healthcare Antiseptics method to test the antimicrobial and hand hygiene effects of tea tree oil disinfectant as shown in previous studies to have exceptional antimicrobial effects with little skin irritation[26] The objective of the present study was to compare the hand disinfection effects of a tea tree essential oil, alcohol and benzalkonium chloride on microbial counts, subjective skin condition and transepidermal water loss (TEWL).

What's known?

- Hand hygiene is a critical factor in preventing healthcare-associated pathogen transmission.
- Hand hygiene also helps stem the spread of infections in the workplace.
- Hand hygiene is a key factor in preventing and managing bacterial or viral epidemics.
- In healthcare settings, hand hygiene performance rates range from a low of 5% to a high of 89%.

What's new?

- Tea tree oil disinfectant is an effective agent for disinfecting hands.
- Hand hygiene formulations that contain tea tree oil may increase the effectiveness of hand disinfection, preventing or reducing healthcare-associated pathogen transmission.

2 | METHODS

2.1 | Study design

A randomised controlled prepost–experimental design was employed to investigate the antimicrobial and hand hygiene effects of a tea tree oil disinfectant. The study population included four groups: (a) the tea tree oil (experimental) group in which subjects applied a tea tree oil disinfectant; (b) the alcohol group in which subjects applied an alcohol-based hand sanitiser; (c) the benzalkonium chloride group in which subjects applied a benzalkonium chloride-based hand sanitiser and (d) the control group in which no disinfectant was applied (Figure 1).

2.2 | Study population

2.2.1 | Subject recruitment

Healthy adult subjects were recruited by posting announcements in community groups and institutions in a metropolitan city. The inclusion criteria were as follows: being aged 18-60 years; being able to fully understand the objectives and procedures of the study and voluntarily consenting to participate. The exclusion criteria were as follows: having a skin disease concerning the hands or forearms; having an open wound; suffering from a hangnail or other skin abnormalities; taking immunosuppressant drugs or other antibiotics and having an adverse reaction to tea tree essential oil ascertained by inquiries and observations.

2.2.2 | Sample size estimation

The sample size for the study was estimated using G-Power 3.1.9.2 software. Since there was no precedent established regarding effect size, a moderate effect size of 0.34 was used for the calculation. The calculation parameters were four groups, an α value of 0.05 and a statistical power of 0.80, which resulted in a sample size estimate of 100. Assuming a dropout rate of ~10%, the appropriate sample size was determined to be 112. Accordingly, 28 participants were randomly assigned to each of the four groups. The final study population consisted of 106 participants: 26 in the tea tree oil group, 27

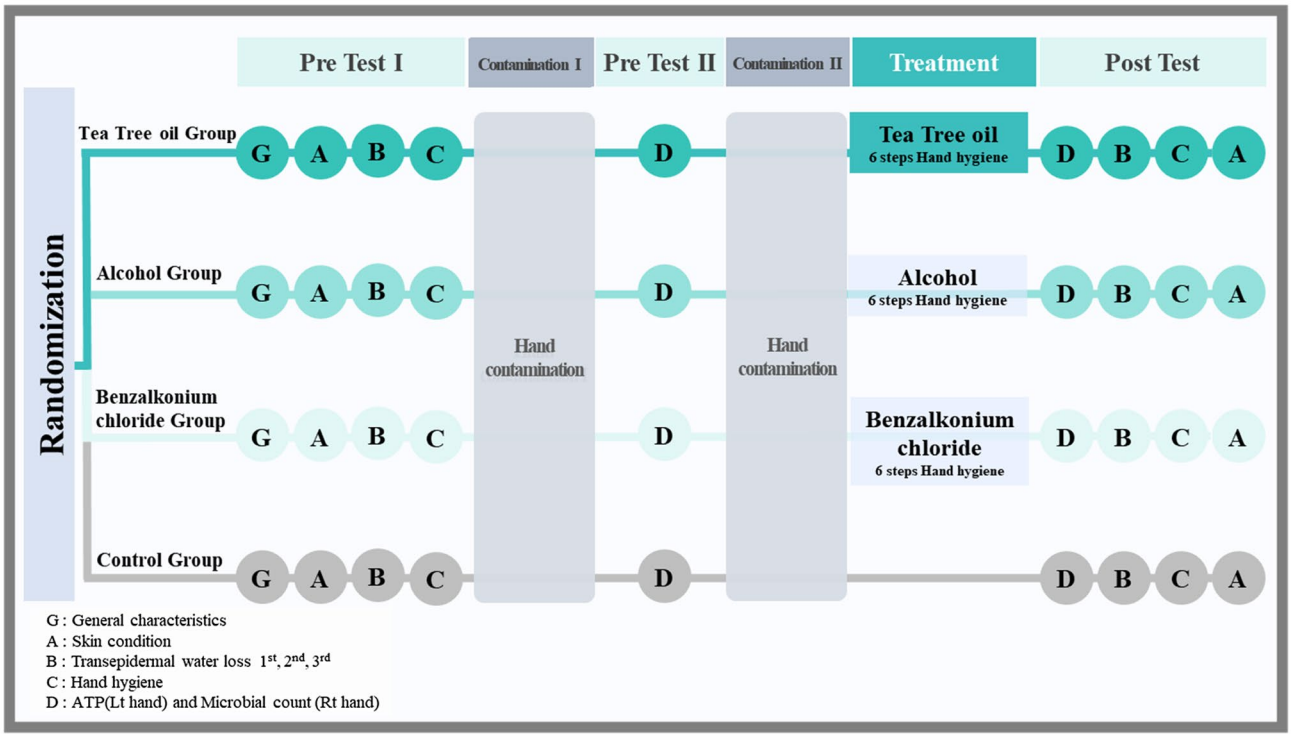

FIGURE 1 Study design

in the alcohol group, 26 in the benzalkonium chloride group and 27 in the control group (Figure 2).

2.2.3 | Participant allocation and concealment

To prevent allocation bias, the participants were codified according to the order of enrolment. They were then randomly allocated to one of the four groups based on allocation results generated by an Excel function. Moreover, this was a double-blind study; neither the participants nor the research assistants had any knowledge of the experimental group.

2.3 | Outcome measures

2.3.1 | Homogeneity testing

A self-reporting questionnaire on participants' demographic factors was administered to enable homogeneity testing across the four groups.

2.3.2 | Primary outcomes

Adenosine triphosphate

Adenosine triphosphate (ATP) was evaluated using the ATP Surface Test kit and a Clean-Trace Luminometer (3 M Health Care), which assesses the presence of surface organisms using bioluminescence. The value was based on the light emitted when the reagent from the ATP Surface Test kit reacted with adenosine triphosphate from organic materials. A cotton swab from the test kit was used to draw a square (~10 cm × 10 cm) on the centre of the left palm, after which the surface inside the square was rubbed in one direction, followed by rubbing in the opposite direction. The cotton swab was then placed inside the test kit tube which was shaken vigorously to ensure mixture with the reagent. After side-to-side shaking for at least 5 seconds, the tube was placed inside the Clean-Trace Luminometer to measure adenosine triphosphate values in relative light units (RLU), with higher values indicating higher levels of contamination.

Microbial count

To measure the number of microbial colonies on a subject's hands, the glove juice sampling procedure, following the US FDA-Tentative Final Monograph for Healthcare Antiseptics method, was applied using the MacConkey agar plate (Asan, Korea) culture test medium. Both hands were contaminated with marker microorganisms, and after the hands were completely dried, a powder-free sterile latex glove was worn on the right hand and 50 mL of physiological saline was injected into the glove. The glove was sealed at the wrist to prevent the saline from leaking. The researcher carefully massaged the gloved hand for 1 minute. Subsequently, a pipette was used to sample 100 μL of the physiological saline inside the glove. The collected sample was diluted by 10^4, and the MacConkey agar plate was inoculated with the sample. After contaminating both hands with the marker microorganisms

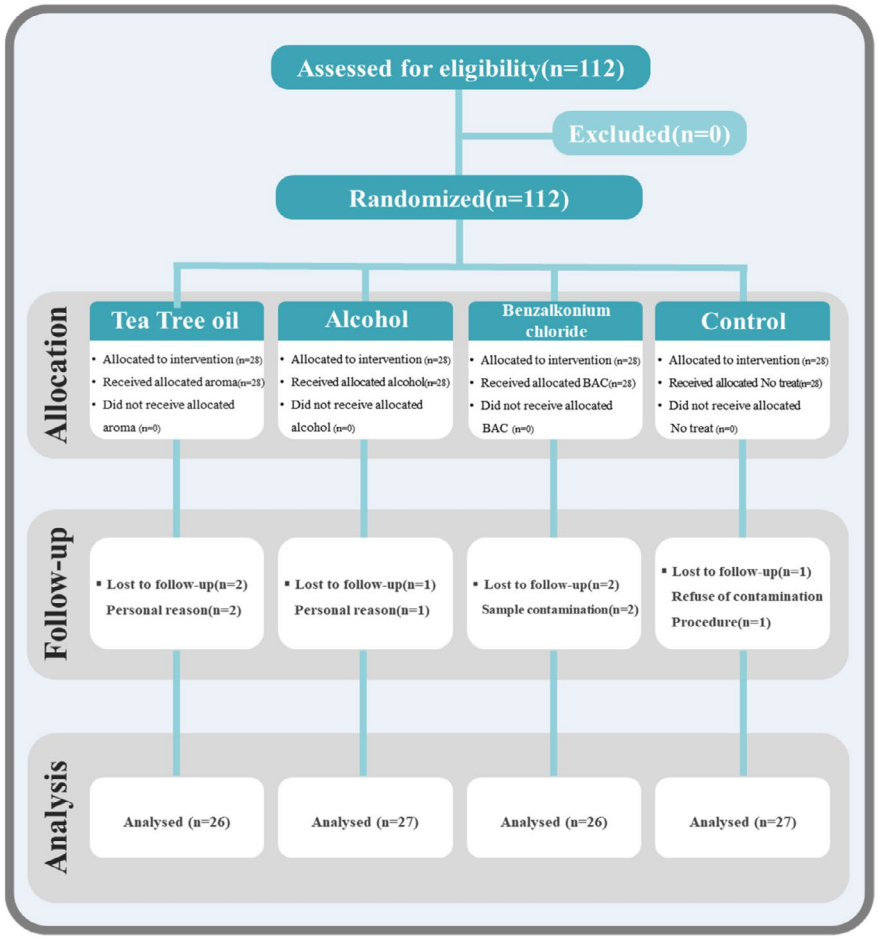

FIGURE 2 Flow diagram of the study groups

again, the subject's hands were completely dried and the experimental treatment was applied. The procedure described was then repeated. The inoculated MacConkey agar plate was placed in a 25℃ incubator for 48 hours. If the level of the marker microorganisms decreased by 2-$\log_{10}$ within 5 minutes of the first hand disinfection, the hand sanitiser was determined to have had an antimicrobial effect.

2.3.3 | Secondary outcomes

Subjective skin condition

Subjective skin condition was assessed by three items (skin moistness, skin dryness and skin exfoliation) on a 5-point Likert scale (1 = not at all, 5 = extremely high).

Transepidermal water loss

TEWL assessed by applying the gpskin Barrier probe (gpower, Korea) and analysing TEWL using the gpskin application, which is a dedicated analysis program for the gpskin Barrier. The probe was placed perpendicular to the centre of the dorsal side of the left hand for three consecutive TEWL measurements, the mean of which was used in the analysis. The gpskin Barrier analysis unit of TEWL was g/m^2/hr A normal range was within 16-20 g/m^2/hr Higher values indicated greater water loss.

2.4 | Experimental treatment

2.4.1 | Preparation and application of disinfectant

The experimental treatments consisted of a tea tree oil disinfectant (the tea tree oil group), an alcohol-based hand sanitiser (the alcohol group) and a benzalkonium chloride-based hand sanitiser (the benzalkonium chloride group). In addition to these experimental treatment groups, the study also included a control group that received no treatment.

1. The tea tree oil group used a tea tree oil solution prepared under the supervision of an international aromatherapist with extensive research experience. Tea tree oil, solubiliser, glycerin and sterile distilled water were mixed in a ratio of 2:2:1:15 to develop the 10% tea tree oil disinfectant. It was stored in a refrigerator with the temperature maintained at 2℃-8℃ before use. As per the American Society for Testing and Materials E 1174 standard testing method, the sample was placed in a 5-mL syringe and dispensed in three doses.
2. The alcohol group used a gel-type hand sanitiser without water; 2 mL (equivalent to two pumps) of the sanitiser contained 83% alcohol (Sanisara W, SARAYAKOREA, Korea), which was commonly used in medical institutions, was applied to the subjects' hands.

3. The benzalkonium chloride group used a foam-type hand sanitiser without water; 0.8 mL (equivalent to one pump) of benzalkonium chloride (NewGenn Foam Hand Rub, NewGenn Science, Korea), commonly used as an alcohol-free hand sanitiser in medical institutions, was applied to the subjects' hands.
4. Control group received no treatment.

2.5 | Study procedures

2.5.1 | Data collection

Data collection took place between April 9 and April 13, 2018.

2.5.2 | Marker microorganism preparation

1. *Serratia marcescens* (ATCC 14756), an experimental microorganism, designated by the FDA-Tentative Final Monograph for Healthcare Antiseptics method for assessing hand hygiene products,[27] was procured from the Korean Culture Center of Microorganisms.
2. Identifying the gentamicin susceptibility of marker microorganisms: The subjects were instructed on how to receive treatment in the event of any problems associated with the application of the marker microorganisms to the skin. Moreover, adopting the FDA-Tentative Final Monograph for Healthcare Antiseptics method, a gentamicin susceptibility test[27] was performed to provide clinicians with information about the antibiotic susceptibility of *Serratia marcescens* (ATCC 14756) in the event of skin infection. The results confirmed susceptibility to gentamicin and 14 other types of antibiotics.
3. Culturing and applying marker microorganisms: After inoculating soybean-casein digest broth with *Serratia marcescens* (ATCC 14756), culturing was carried out for 24 hours at 25℃. Following the American Society for Testing and Materials 1174 standard testing protocol,[28] the sample was prepared in a concentration of 1×10^8 CFU/mL. Each *Serratia marcescens* solution prepared according to the standard was used for no more than 8 hours.

2.5.3 | Application of hand hygiene method

For the hand hygiene method, the "How to handrub" illustration presented in the 2009 WHO hand hygiene guidelines[29] was modified accordingly to satisfy the experimental procedure employed in this study. The illustration was posted in the hand cleaning area so that all subjects could clean their hands using the same method. When applying the experimental treatment, the assigned disinfectant was dispensed into the subjects' cupped hands and the subjects were instructed to thoroughly and evenly rub their palms, the tops of their hands, in between their fingers, and their fingers, thumbs and fingernail tips for at least 30 seconds.

2.5.4 | Pilot experiment

Four participants were selected and each was assigned to one of the groups (tea tree oil, alcohol, benzalkonium chloride and control). After applying 5 mL of the marker microorganism, *Serratia marcescens* (ATCC 14756), to contaminate both hands, the glove juice sample procedure was conducted. After contaminating both hands again with the marker microorganism, each of the treatment group pilot participants was instructed to disinfect their hands with their assigned disinfectant (tea tree oil disinfectant, alcohol-based hand sanitiser or benzalkonium chloride-based hand sanitiser). No treatment was given to the control group. The glove juice sample procedure was repeated. The complete experimental procedure was checked through this process, and since it was determined that there was a risk of the marker microorganism contaminating the participants' clothing, each participant was provided with a disposable vinyl gown.

2.5.5 | Pretest I prior to the experimental treatment

Upon visiting the laboratory, the subjects were informed about the objectives, procedures and precautions regarding the study, after which, they provided written informed consent and completed a questionnaire on their general characteristics and skin condition. Next, three consecutive TEWL measurements were taken. Each participant was provided with a disposable vinyl gown to wear and instructed to wash their hands with water and a liquid soap containing no disinfectants. The subjects' hands were dried with paper towels, and the used paper towels were disposed of as medical waste.

2.5.6 | Marker microorganism application I

To contaminate the subjects' hands, 5 mL of *Serratia marcescens* in a concentration of 1×10^8 CFU/mL was placed in a pipette and dispensed below both wrists in three doses, as previously described in the Methods section. The participants were instructed to rub it evenly on both hands for 1 minute and allow their hands to air dry for 2 minutes.

2.5.7 | Pretest II prior to the experimental treatment

Adenosine triphosphate was measured to determine the degree of hand contamination, as described in the Methods section. Besides, the number of microbial colonies on the subjects' hands was measured using the glove juice sampling procedure also previously described in the Methods section. The microbial count was calculated based on the dilution factor used.

2.5.8 | Marker microorganism application II

The subjects' hands were contaminated a second time, as described in the Methods section, using 5 mL of *Serratia marcescens* placed in a

pipette and dispensed below both wrists in three doses. The subjects were instructed to rub it evenly on both hands for 1 minute and allow them to air dry for 2 minutes.

2.5.9 | Application of the experimental treatment

The experimental treatments described in the Methods section were applied to each group: a tea tree oil disinfectant for the tea tree oil group, an alcohol-based hand sanitiser for the alcohol group, a benzalkonium chloride-based hand sanitiser for the benzalkonium chloride group and no treatment for the control group.

2.5.10 | Posttest after the experimental treatment

1. To identify pre- and posttreatment differences in adenosine triphosphate values and microbial counts, adenosine triphosphate measurements and microbial culture tests were performed using the same methods as in the pretest conducted before the experimental treatment.
2. Three consecutive measurements of TEWL were taken to identify pre- and posttreatment changes in skin condition.
3. To eliminate any marker microorganisms remaining on the skin, soap and water were used to wash the subjects' hands and forearms for at least 1 minute. After drying with paper towels, hand sanitiser comprising 83% ethanol was used for hand hygiene.
4. A questionnaire on subjective skin condition was completed to identify changes in posttreatment skin conditions.

2.5.11 | Safety assurance for managing marker microorganisms

Marker microorganisms utilised to assess hand disinfection effects were applied per the hand hygiene guidelines for medical institutions adopted from the FDA-Tentative Final Monograph for Healthcare Antiseptics method and the Centers for Disease Control.[30] For the subjects' safety, all participants were asked about any skin diseases, open wounds, hangnails or other skin abnormalities when recruited for the study, and they were visually rechecked before starting the experiment.

For the safety of the research assistant, a disposable vinyl gown and latex gloves were supplied when participating in the experimental treatment. After each experiment, the assistant performed hand hygiene using an alcohol-based hand sanitiser or soap and water.

To prevent cross-infection, after each subject's participation, disinfectant wipes were used to disinfect the laboratory environment and any equipment which the subject may have contacted.

2.6 | Ethical considerations

The researcher completed an online education course on the KCDC Bioethics and Safety Act in January 2018. The research protocol was submitted to the University IRB, where it was reviewed and approved (EU18-15). The subjects were also informed about the right to withdraw their consent at any time during the study. Collected data were assigned unique numbers and managed according to personal information processing guidelines for the protection of personal information.

2.7 | Data analysis

The collected data were analysed using IBM SPSS Statistics, version 24.0. A homogeneity test on the study population was performed using χ^2 tests, Fisher's exact tests and ANOVA. The pre- and posttreatment differences in the adenosine triphosphate $\log_{10}$ values, microbial count $\log_{10}$ values, TEWL and subjective skin condition in all four groups were analysed using ANOVA with Scheffé post hoc tests. Additionally, paired *t*-tests were used to examine the pre- and posttreatment group differences concerning subjective skin condition.

3 | RESULTS

3.1 | Subject characteristics and pre-experimental homogeneity testing

The subjects in the present study were randomly allocated to four groups: tea tree oil (n = 26), alcohol (n = 27), benzalkonium chloride (n = 26) and control (n = 27). The general characteristics or variables of the four groups and homogeneity test results are shown in supplementary file 1. The homogeneity tests showed no significant between-group differences in age, gender and education level; therefore, homogeneity among the four groups was confirmed.

3.2 | Treatment effects on primary outcomes

Table 1 and Figure 3A show the adenosine triphosphate $\log_{10}$ values measured before and after experimental treatment, determining the hand disinfection effect of the tea tree essential oil disinfectant.

The adenosine triphosphate $\log_{10}$ values before and after the experimental treatment were $\log_{10}$ 4.38 RLU and $\log_{10}$ 3.92 RLU, respectively, in the tea tree oil group; $\log_{10}$ 4.41 RLU and $\log_{10}$ 4.00 RLU, respectively, in the alcohol group; $\log_{10}$ 4.50 RLU and $\log_{10}$ 4.08 RLU, respectively, in the benzalkonium chloride group; $\log_{10}$ 4.63 RLU and $\log_{10}$ 4.52 RLU, respectively, in the control group.

There were no statistically significant differences in the mean adenosine triphosphate $\log_{10}$ values when the marker microorganism *Serratia marcescens* was applied to both hands before the experimental treatment and isolated for measurement. The results indicated that the subjects in all four groups had the same degree of contamination on their hands prior to the experimental treatment. However, significant differences in the mean adenosine triphosphate $\log_{10}$ values were found after the experimental treatment

TABLE 1 Comparison of ATP log_{10} and microbial count among four groups (N = 106)

Variable	Handrub Formulations	n	Baseline ATP (Pre) M ± SD (log_{10}) (95% CI)	Residual ATP (Post) M ± SD (log_{10}) (95% CI)	Difference (Prepost) M ± SD (log_{10}) (95% CI)
ATP log_{10}	Tea tree oil Group	26	4.38 ± 0.50 (4.18-4.59)	3.92 ± 0.27[a] (3.81-4.03)	0.46 ± 0.51[a] (0.26-0.67)
	Alcohol Group	27	4.41 ± 0.50 (4.21-4.61)	4.00 ± 0.39[a] (3.84-4.16)	0.41 ± 0.50[a] (0.21-0.61)
	Benzalkonium chloride Group	26	4.50 ± 0.51 (4.29-4.71)	4.08 ± 0.27[a] (3.97-4.19)	0.42 ± 0.50[a] (0.22-0.62)
	Control Group	27	4.63 ± 0.49 (4.43-4.82)	4.52 ± 0.51[b] (4.32-4.72)	0.11 ± 0.32[b] (−0.02-0.24)
	F		1.33	13.47	3.23
	P		0.270	<0.001	0.025
Microbial count	Tea tree oil Group	26	6.81 ± 0.63 (6.55-7.06)	1.31 ± 2.02[a] (0.49-2.12)	5.50 ± 1.90[a] (4.73-6.27)
	Alcohol Group	27	6.96 ± 0.44 (6.79-7.14)	4.63 ± 1.52[b] (4.03-5.23)	2.33 ± 1.62[b] (1.69-2.97)
	Benzalkonium chloride Group	26	7.08 ± 0.48 (6.88-7.27)	6.46 ± 0.65[c] (6.20-6.72)	0.62 ± 0.57[c] (0.38-0.85)
	Control Group	27	6.78 ± 0.70 (6.50-7.05)	6.85 ± 0.72[c] (6.57-7.14)	-0.07 ± 0.73[c] (−0.36-0.21)
	F		1.57	92.14	91.71
	P		.203	<.001	<.001

Note: Means for each group with different superscript (a, b, c) indicate a significant difference (Scheffe's test; $P < .05$).
Abbreviations: ATP, Adenosine triphosphate; M ± SD, Mean ± standard deviation.

(F = 13.47, $P < .001$), with significant differences in the amount of change: log_{10} 0.46 RLU, log_{10} 0.41 RLU, log_{10} 0.42 RLU and log_{10} 0.11 RLU in the tea tree oil, alcohol, benzalkonium chloride and control groups, respectively (F = 3.23, $P = .025$).

Table 1 and Figure 3B show the microbial count log_{10} values measured to identify the hand disinfection effect of the tea tree essential oil disinfectant. The microbial count log_{10} values before and after the experimental treatment were log_{10} 6.81 and log_{10} 1.31, respectively, in the tea tree oil group; log_{10} 6.96 and log_{10} 4.63, respectively, in the alcohol group; log_{10} 7.08 and log_{10} 6.46, respectively, in the benzalkonium chloride group; and log_{10} 6.78 and log_{10} 6.85, respectively, in the control group.

There were no statistically significant differences in the mean microbial count log_{10} values when the marker microorganism *Serratia marcescens* was applied to artificially contaminate both hands before the experimental treatment and isolated for measurement. The results indicated that the subjects in all four groups had the same degree of contamination on their hands prior to the experimental treatment. However, significant differences in the mean microbial count log_{10} values were found after the experimental treatment (F = 92.14, $P < .001$), with significant differences in the amount of change: log_{10} 5.50 ± 1.90, log_{10} 2.33 ± 1.62, log_{10} 0.62 ± 0.57 and log_{10} −0.07 ± 0.73 in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively (F = 91.71, $P < .001$).

3.3 | Treatment effects on secondary outcomes

Figure 3C shows the results of skin moistness measured to determine the effects of the tea tree essential oil disinfectant on skin condition. There were no statistically significant differences in skin moistness among the four groups before the experimental treatment, with 2.65, 2.41, 2.35 and 2.59 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. The results also showed no significant differences in skin moistness among the four groups after the experimental treatment, with 3.62, 3.04, 3.31 and 3.19 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. However, there were statistically significant differences in the amount of change between before and after the experimental treatment, with 0.96, 0.63, 0.96 and 0.59 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively (Supplementary file S2).

Figure 3D shows the results for skin dryness. There were no significant differences in skin dryness among the four groups before the experimental treatment, with 3.35, 3.52, 3.58 and 3.44 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. The results also showed no significant differences in skin dryness among the four groups after the experimental treatment, with 2.54, 3.26, 2.73 and 2.85 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively.

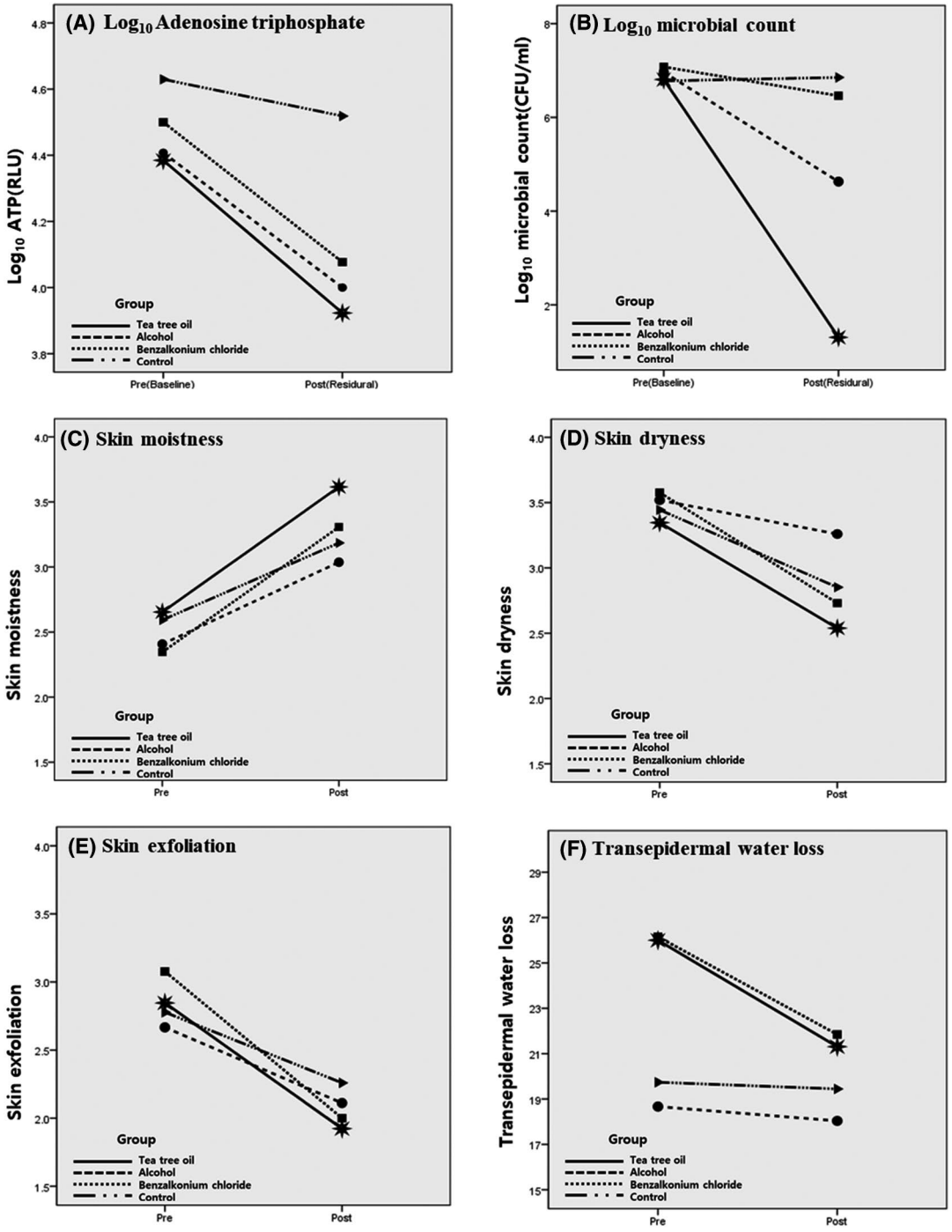

FIGURE 3 Comparison of variables among four groups

However, there were statistically significant differences in the amount of change between before and after the experimental treatment in each group, with −0.81, −0.26, −0.85 and −0.59 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively (Supplementary file S2).

Figure 3E shows the results for skin exfoliation. There were no significant differences in skin exfoliation among the four groups before the experimental treatment, with 2.85, 2.67, 3.08 and 2.78 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. The results also showed no significant differences in skin exfoliation among the four groups after the experimental treatment, with 1.92, 2.11, 2.00 and 2.26 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. Moreover, there were no significant differences in the amount of change between before and after the experimental treatment in any group, with −0.92, −0.56, −1.08 and −0.52 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively (Supplementary file S2).

In sum, there were no significant differences in skin moistness, skin dryness or skin exfoliation among the four groups after the experimental treatment.

Figure 3F shows the results of measurements determining the effects of the tea tree essential oil disinfectant on TEWL, which before the experimental treatment measured 26.00, 18.67, 26.15 and 19.74 in the tea tree oil, alcohol, benzalkonium chloride and control groups, respectively, indicating no statistically significant differences (Supplementary file S2).

The results also showed no statistically significant differences among the four groups after the experimental treatment, with 21.31, 18.04, 21.85 and 19.44 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively. Moreover, there were no significant differences in the amount of change before and after the experimental treatment, with −4.69, −0.63, −4.30 and −0.30 measured in the tea tree oil, alcohol, benzalkonium chloride and control groups respectively.

4 | DISCUSSION

The present study aimed to investigate the antimicrobial and hand hygiene effects of a tea tree essential oil disinfectant on healthy adults and provide basic data for utilising tea tree essential oil disinfectant as a nursing intervention.

4.1 | The effects of the tea tree essential oil on hand disinfection

The present study was conducted to identify the hand disinfection effects of tea tree oil disinfectant, which is known to have antimicrobial effects. To contaminate the subjects' hands prior to the experimental treatment, *Serratia marcescens* (ATCC 14756) was used in accordance with the FDA-Tentative Final Monograph for Healthcare Antiseptics method. After contaminating the subjects' hands, adenosine triphosphate values and microbial counts were obtained before and after the experimental treatment, and both values were analysed after conversion to log values to reduce the deviation in values.

The results showed similar decreases in the adenosine triphosphate values of the tea tree oil, alcohol and benzalkonium chloride groups after the experimental treatment, while extremely low decreases were found among the control group. Moreover, the tea tree oil group showed the largest decrease and the alcohol group showed a moderate decrease, whereas the benzalkonium chloride and control groups showed no significant decreases after treatment. Based on these findings, it was determined that the tea tree oil and alcohol groups showed appreciable decreases in adenosine triphosphate values and microbial counts, whereas the benzalkonium chloride and control groups exhibited no significant changes.

As the degree of contamination measured by adenosine triphosphate tests includes dead microorganisms, the method has limitations concerning the identification of decreases in microbial counts. Moreover, there are no set of standards for adenosine triphosphate values, so individual determinations are made based on target values set according to basic guidelines recommended by each manufacturer. Therefore, it is necessary to view microbial counts as more meaningful than adenosine triphosphate values. From that perspective, the tea tree oil group showed a more significant decrease in microbial counts than the alcohol group, while the benzalkonium chloride and control groups showed no significant differences in microbial counts. Thus, it was determined that the tea tree oil group demonstrated the highest antimicrobial effect, followed by the alcohol group, whereas the benzalkonium chloride and control groups revealed little antimicrobial effect based on microbial counts.

Although a previous study[31] reported that high adenosine triphosphate values indicate high microbial counts, they may also indicate high levels of organic materials, including dead microorganisms; therefore, adenosine triphosphate values have limited value for identifying disinfection effects. However, high adenosine triphosphate values also indicate a favourable environment for bacterial growth; hence, adenosine triphosphate values could be used for evaluating visible effects and providing immediate feedback on disinfection effects.

Considering microbial counts, the tea tree oil group showed an appreciably higher disinfection effect, whereas the benzalkonium chloride group exhibited no disinfection effect based on adenosine triphosphate measurements, with the control group demonstrating similar results. These findings were consistent with a study reporting that hand hygiene using benzalkonium chloride-based wipes did not satisfy the microbial count log decrease value for *Serratia marcescens*.[32] The findings were also consistent with another study reporting that disinfection effects were unsatisfactory for infections caused by benzalkonium chloride-based disinfectant contamination.[33] As studies have reported benzalkonium chloride to be more effective for disinfection of gram-positive bacteria but limited for disinfecting gram-negative bacteria,[34,35] the findings in the present study may have resulted from using gram-negative bacteria as the artificial contamination source.

In particular, considering that tea tree essential oil contains terpinen-4-ol and 1,8-cineole, a component known to have antimicrobial effects, the present study's findings were consistent with previous studies reporting that tea tree essential oil disinfectants have a disinfection effect,[21,36] demonstrating that the disinfection effect of the tea tree oil group was comparable with that of the alcohol group. However, as the proportion of tea tree essential oil was extremely high, future studies are necessary to identify the optimal proportion, while studies on cost-effectiveness, ease of use and economic feasibility are also required.

4.2 | The effects of the tea tree essential oil disinfectant on skin condition

Skin moistness, skin dryness and skin exfoliation were measured before and after the experimental treatment to identify the effects of

the tea tree essential oil disinfectant on skin condition. The results showed no significant differences among the four groups regarding skin moistness, skin dryness and skin exfoliation after the experimental treatment.

Based on a review of previous studies, tea tree essential oil is generally believed to have a skin-protective effect, and the glycerin contained in tea tree disinfectant solution also influences skin moisture[37]; therefore, increased subjective skin moisture and reduced TEWL were expected to be observed. However, the findings in this present study did not support this. Since all four groups showed significant increases in skin moistness following the experimental treatment, it is possible that the marker microorganism solution, which was submerged in soybean-casein digest broth, may have contributed to increased skin moistness in the posttreatment tests. Moreover, individual differences in skin moisture levels and the effects of the contamination treatment and experimental treatment on the skin could all be confounding variables; however, this could not be confirmed in the study. To identify effects on skin condition, it would be necessary to apply the treatment for a longer duration and determine skin condition before and after the application.

5 | CONCLUSION

In conclusion, the tea tree essential oil disinfectant was demonstrated to have antimicrobial and hand disinfection effects, but positive effects on the skin could not be confirmed. Given these findings, tea tree oil disinfectant could be utilised in nursing as a new hand hygiene formulation.

The implications of the present study are twofold. First, most of the previous studies on the antimicrobial effects of tea tree essential oil were conducted in laboratory settings and few studies examined disinfection effects on actual skin. In the present study, disinfectant was applied to the skin, providing a much more sensitive and effective test of differences in effects, characterising them using various organic materials and based on various microorganisms and contaminants attached to the skin. Consequently, it is recommended that tea tree oil disinfectants be employed as a new hand hygiene formulation. Second, confirming the hand disinfection effects of tea tree oil disinfectant allows for different concentrations, compositions and application methods to be developed and used in the future.

We recommend that future research on tea tree oil disinfectant applies different blending ratios and essential oil volumes in repeated studies. Furthermore, to better identify any potential skin effects, future studies should eliminate confounding variables by increasing the volume of moisturiser and extending the duration of treatment.

STUDY REGISTRATION NUMBER

Registered with Clinical Research information Service (CRIS) (KCT0003240): CRIS is an online registration system for clinical trials and clinical research conducted in Korea, and has joined the WHO International Clinical Trials Registry Platform (ICTRP) as the 11th Registry in the world.

CONFLICT OF INTEREST

The authors declare there was no conflict of interest.

AUTHOR CONTRIBUTIONS

Bock-Hui Youn: The conception and design of the study, acquisition of data, data analysis and manuscript correction. **Yeon-Suk Kim**: Data analysis, interpretation of data and manuscript correction. **Seungmin Yoo**: Interpretation of data and manuscript correction. **Myung-Haeng Hur**: The conception and design of the study, data analysis, interpretation of data and manuscript correction. All authors approved the final manuscript.

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