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Antimicrobial Effect of Deodorant Products Containing *Rhinacanthus nasutus* Extract for Reducing Armpit Odor

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Abstract

The aim of this study was to investigate the effectiveness of developing a roll-on deodorant that can reduce armpit microbiota bacteria to decrease armpit odor. The richest apocrine sweat glands in the armpit region secrete a variety of odor precursors that are transformed into volatile odoriferous substances by bacterial enzymes on the skin surface. The dominant armpits microbiota included four groups of bacteria such as *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and Propionibacterium spp., and also fungi or yeasts. Two formulas of the roll-on deodorant products, RDEOF-1 and RDEOF-2 were developed. RDEOF-2 contained an ethanolic extract of *Rhinacanthus nasutus* (L.) Kurz leaves inhibited several microorganisms, including Gram-positive and Gram-negative bacteria. Sixteen healthy volunteers showed satisfactory assessment for RDEOF-2 that was greater than RDEOF-1. Armpit bacteria were collected by swab method and armpit odor was evaluated by ASTM method. Results showed dominant bacteria of two genera including *Staphylococcus* spp. and *Corynebacterium* spp. in all swab samples that mainly cause armpit odor. Armpit bacterial numbers before using deodorants were high in the range of $2x10^3$ to $9x10^5$ (CFU/mL). After applying RDEOF-1 and RDEOF-2, bacterial numbers decreased in the range of 1x103 to $8x10^5$ and $3x10^2$ to $4x10^5$ (CFU/mL), respectively. Armpit bacteria were found in males more than in females. Deodorant products containing *R. nasutus* extract have been shown to reduce the bacteria that cause armpit odor. Therefore, the development of the deodorant product with natural plant extracts is warranted.

Introduction

The human body can disseminate odorous substances with the breath, saliva, sweat (from skin), urine, or reproductive organs fluids (Mogilnicka et al., 2020). The major odorants are small, volatile compounds that may either be produced in situ such as skin and oral cavity, or be carried by blood circulation from the gut, which is the main site of bacterial metabolism. Skin is the largest, most functional organ of the human body. A

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complex mixture of hundreds of chemical compounds (carboxylic acids, ketones, aldehydes, alcohols, esters, hydrocarbons, etc.), are transformed from precursors secreted by excretory skin glands (Wilke et al., 2007; Ferdenzi et al., 2020). The human armpit is covered by a dense array of glands, and the area of this skin niche is colonized by a large population of bacteria or microbiome (Wilke et al., 2007; Natsch & Emter 2020). Also, the armpit has a major function in human body odor formation. The richest apocrine sweat glands in the armpit region secrete a variety of odor precursors that are transformed into volatile odoriferous substances by bacterial enzymes on the skin surface (Natsch et al., 2005; Natsch & Emter 2020). The main contributors to armpits odor are a group of acids, with 3-methyl-2-hexenoic acid (3M2H) and 3-hydroxy-3-methyl-hexanoic acid (HMHA) and also a group of sulfanylalkanols, particularly 3-methyl-3-sulfanylhexan-1-ol (3M3SH) (Troccaz et.al., 2004; Martin et al., 2010). Bromhidrosis, which is also known as osmidrosis or malodorous sweating, is a distressing condition that is characterized by offensive body odor, noticeable especially in the armpits, genital, or feet area. All three types of sweat glands (apocrine, eccrine, and apoeccrine) play a role in the pathogenesis of this disease. Excessive sweating followed by decomposition of sweat constituents by bacteria results in an unpleasing smell of sweat (Semkova et al., 2015; Mogilnicka et al., 2020).

The dominant armpits microbiota inhabiting skin surface break down apocrine sweat into numerous volatiles molecules such as ammonia and short-chain fatty acids including four groups of bacteria such as Staphylococcus, Micrococcus, Corynebacterium, and Propionibacterium, and also fungi or yeasts such as Malassezia and Candida (Leaden et al., 1981; Wilke et al., 2007; Mogilnicka et al., 2020; Moskovicz et al., 2020; Ogbebor 2021). Deodorants and antiperspirants have been used for centuries worldwide, evolving from simple fragrances that masked aggressive odors to complex ingredients based on aluminum and zirconium chemistries that act to slow or diminish sweat production, or botanical extracts with antimicrobial activity. They help to degrade apocrine axillary secretions while antiperspirants function by reducing armpit odor and perspiration levels from the armpit making it dry and comfortable (Philip & Jack 2000; Abrutyn, 2010; Ogbebor 2021; Abrutyn 2022). Roll-on deodorant usually comes in form of a glass or plastic bottle with a ball top. The product is applied by rolling the ball over the skin and leaving a wet sensation behind the application. (Gámbaro et al., 2019).

Deodorant manufacturers have developed a variety of products employing these mechanisms including the physical methods, such as the use of silica gel or activated carbon to absorb malodorous compounds; biological methods, such as the use of sanitizers that inactivate malodor-producing bacteria; chemical methods, which involve chemical inactivation of malodorous compounds; and for sensory methods, which involve masking unpleasant odors with fragrances. Nowadays botanical extracts play an important role in deodorant products, green tea catechins are widely used in Japan and Southeast Asia. (Henmi et al., 2020). Thailand has many medicinal plants such *Rhinacanthus nasutus* (L.) Kurz that contain rhinacanthin showed the effect of inhibiting many microorganisms including Gram-positive bacteria such as *Staphylococus aureus,* Gram-negative bacteria such as *Escherichia coli*, and fungi such as *Candida albicans* (Puttarak et al., 2010; Kumar et al., 2021, Onvimol et al. 2021). Our previous study showed that *R. nasutus* extract had bacterial growth inhibitory activity and, especially, this extract inhibited *S. aureus, S. epidermidis* and *Escherichia coli* that were found on human skin (Panichakul et al., 2017). In our previous study, *R. nasutus* extract was used to apply in the development of liquid soap and alcohol gel as presented in the petty patent (no. 1503001746 and no. 1803000589). The present study aimed to investigate the effectiveness of developing roll-on deodorant to reduce armpit microbiota bacteria in order to decrease the armpit odor.

Materials and methods

1. Participants

Sixteen healthy volunteers (male and female) aged 19-50 years were enrolled after they provided their consent to participate in this study. All volunteers had no history of allergic skin rash, no wound on the skin. Reasons for exclusion were pregnancy, hypersensitivity to cosmetics and skin diseases. The ethical and methodological aspects of this study on volunteers for odor evaluation and bacterial collection (SDU-RDI_HS 2021-002) have been approved by Ethical Review Subcommittee for Human Research in Health Science, Research and Development Institute, Suan Dusit University, Bangkok, Thailand.

2. Roll-on deodorant formulations

Two formulas of the roll-on deodorant product were developed and provided by Siam Natural Products Co.,Ltd. Khet Phaya Thai, Bangkok. Roll-on deodorant formula-1 and 2 (RDEOF-1 and RDEOF-2) were prepared with the same base formula which contained water, butylene glycol, glycerin, disodium EDTA, steareth-2, ceteareth-12, stearyl alcohol, ceteareth-20, distearyl ether, menthol, tocopheryl acetate, aluminum chlorohydrate, alum, L-glutathione, hydrolyzed collagen, niacinamide, ethyl ascorbic acid, *Aloe barbadensis* leaf extract, and sodium benzoate. The difference between RDEOF-1 and RDEOF-2 was that RDEOF-2 contained 1% (w/w) of ethanolic extract of *Rhinacanthus nasutus* (L.) Kurz. For preparing *R. nasutus* extract modified from a method previously described (Panichakul et al., 2017). Leaves of *R. nasutus* were dried and extracted in 95% ethanol for 7 days at room temperature and evaporated under reduced pressure below 45°C.

3. Study design, bacterial sampling, application of formulations and odor evaluation

Briefly, participants discontinued their habitual application of deodorants, antiperspirants, or other cosmetic products to the armpits within 1 week before their first visit to the researcher (day 0) and throughout the entire trial period. At baseline, first time (day 0), the participants' right armpit cavern was swabbed by rotating with a cotton swab that was soaked in 0.85% sodium chloride repeatedly using the side of the swab for 30 s. (Capone et al., 2011; Moskovicz et al., 2020). At the same time, the participants' left armpit was odor tested using a cotton bud held in the armpit for 5 min and then an assessment of armpit malodor was evaluated by 4 investigators, and scoring 1-11 range of odor (0=no malodor, 5=moderate malodor, 10=extremely strong malodor) followed by ASTM method (E 1207-87 standard practice for the sensory evaluation of axillary deodorant) (Dumas et al., 2009). The sample was carried in transfer culture or trypticase soy broth (TSB) and kept at 4°C until analyzed. For deodorant testing, participants received the RDEOF-1 or RDEOF-2 application, and 1 ml of roll-on was used to apply on both right and left armpit in the morning on day 1 and after showering on the next day. On day 3, 48 h after roll-on applying, the armpit malodor was assessed and a swab sample was also collected. For RDEOF-2 testing, the same application protocol was done but RDEOF-2 was applied on day 6 and after 48 h, the armpit malodor was assessed and a swab sample was also collected. All investigators and participants were blinded. The participants were asked by questionnaire: (i) whether they had observed a difference in the odor from the armpit before and after using the products and, if so, (ii) which armpit they considered being less odorous. The assessment by each participant was analyzed to compare between 2 formulas of roll-on products.

4. Microbiology: Quantification and identification of bacteria

Bacteria on the armpit were determined by total aerobic plate count. In brief, 100 µl of each sample from the armpit swab were diluted with normal saline in 10-fold dilution (10^{-1} to 10^{-4}) and each dilution was cultured on trypticase soy agar (TSA) in triplicate plates. The culture plates were incubated in ambient air for 24 h at 35°C. Bacterial growth was estimated semi-quantitatively as 0 (no growth), $1x10^2$, $1x10^3$, $1x10^4$ and $\geq 1x10^5$ CFU/ml. For identification of *Staphylococcus* spp., bacteria were cultured on selective media Baird-Parker agar (BPA), while *Corynebacterium* spp. typical colony was observed on TSA and bacterial morphology was further identified by Gram' staining and was identified under light microscopy, 100X. (Leaden et al., 1981; Troccaz et al., 2009; Yamazaki et al., 2010 ; Capone et al., 2011; James et al., 2013; Urban et al., 2016; Ågren et al., 2020). The individual isolated colony of bacteria was cryopreserved in 10% glycerol at -20°C for future study.

5. Statistical analysis

Data are represented as mean±SD from three biologically independent experiments.

Results and discussion

1. Participants, product application/acceptance, and bacterial swabs

Sixteen healthy volunteers, including 7 males and 9 females (age 19-50 years) were enrolled in during December 2021. Most of the female and no male participants had shaved armpits. Most of the participants had used deodorant and antiperspirant and also one man had never used both deodorant and antiperspirant before. None of the participants withdraw from the study.

Sixteen volunteers were tested by collecting bacterial swabs from the right armpit, while cotton bud insertion under left armpit following ASTM method (Dumas et al., 2009) was used to detect odor. Bacterial samples were cultured and the results of the quantitative bacteriological survey of 16 volunteers are shown in Table 1. Sixteen participant exhibit total numbers of armpit bacteria before using deodorants in a range of 2x10^3 to 9x10^5 (CFU/mL) and armpit bacteria were found in males more than in females. After applying RDEOF-1 for 48 h, bacterial numbers decreased to the range of 1x10^3 to 8x10^5 (CFU / mL). After applying RDEOF-2 for 48 h, bacterial numbers were lower in the range of 3x10^2 to 4x10^5 (CFU/mL), compared to before and after using RDEOF-1. These results showed that *R. nasutus* extract could reduce bacterial growth revealing the synergistically effect of the additives in RDEOF-2. Rhinocanthin of *R. nasutus* extract (Puttarak et al., 2010; Kumar et al., 2021, Onvimol et al. 2021) may be the factor that had the additive effect on bacteria inhibition. From the results in Fig. 1, levels of armpit odor from sixteen participants were reduced after applying RDEOF-1 or RDEOF-2, compared with before using roll-on. The reduction of odor could be caused by ingredients formulated in both products relating to the study evaluated the antimicrobial activity of various multifunctional cosmetic ingredients by Youenou et al. (2022), and also zinc oxide exhibit reduced malodor and bacteria growth (Ågren et al., 2020). This study shows that RDEOF-2 reduces axillary odor more than RDEOF-1, which does not contain *R. nasutus* extract.

Table 1 Prevalence and density of armpit resident microbiota before and after using roll-on deodorants

Volunteer (N=16)	Sex	Total aerobic plate count (CFU / mL)					
		Before use	SD	After use RDEOF-1	SD	After use RDEOF-2	SD
1	M	1x10^5	±1.15	5x10^3	±1.15	1x10^3	±0.50
2	M	2x10^3	±0.50	1x10^3	±1.00	1x10^3	±0.55
3	M	9x10^3	±1.15	9x10^3	±1.50	9x10^3	±1.15
4	M	2.8x10^5	±1.00	2.6x10^5	±1.15	2.4x10^5	±1.10
5	F	8x10^5	±0.55	3x10^3	±0.65	1x10^3	±1.15
6	M	6x10^3	±0.58	8x10^5	±0.55	6x10^3	±0.50
7	F	8x10^3	±1.00	8x10^5	±1.58	4X10^5	±0.59
8	F	6x10^5	±0.55	3.2x10^5	±1.15	3x10^4	±0.55
9	F	5x10^4	±1.15	5x10^4	±0.15	8x10^3	±1.00
10	M	8x10^5	±0.65	2x10^5	±0.55	3x10^2	±0.65
11	F	9x10^5	±0.55	3X10^4	±0.63	3X10^3	±1.15
12	M	3X10^3	±0.50	2X10^3	±1.00	1.5X10^3	±1.00
13	F	3X10^3	±0.05	1.2X10^3	±0.55	1X10^3	±0.05
14	F	3X10^3	±1.15	7.2X10^3	±1.00	1.8X10^3	±1.55
15	F	4X10^5	±1.50	3X10^3	±0.65	1X10^5	±0.15
16	F	5X10^4	±1.55	3X10^5	±0.15	1X10^5	±0.55

2. Microbiology: Bacteria identification

Armpit odor showed a strong smell which may have been caused by volatile fatty acids such as 3-hydroxy-3-methylhexanoic acid (HMHA) and the sulphanylalkanol, 3-methyl-3-sulfanylhexan-1-ol (MSH). HMHA is released from a glutamine conjugate by the action of a zinc-dependent aminoacylase from *Corynebacteria* spp., whereas MSH is derived from a cysteinylglycine-S-conjugate by the action of *Staphylococcus* sp. In addition to the *S. epidermidis* strain, the most abundant microorganisms present in human axilla are *Corynebacterium* spp., possessing the aminoacylase, and *Staphylococcus* spp. possessing C-S lyase activities. These substances are found in human armpits and cause armpit odor as onion-like, cheesy and rancid odor emits armpit odors (Natsch et al. 2004; Troccaz et al. 2004; Natsch et al. 2006).

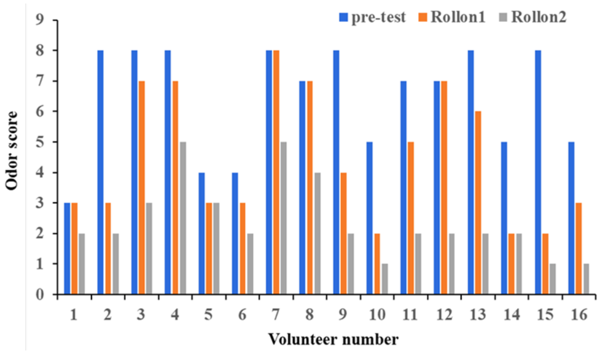

Fig. 1 Armpit odor scores in human volunteers (n = 16) by judges trained for the assessment of axillary malodor before and after the application of RDEOF-1 (Roll-on deodorant formula-1) and RDEOF-2 (Roll-on deodorant formula-2) products

In this study, the typical colony of *Staphylococcus* spp. on BPA was found to be grey-black colonies and a halo, opaque halo, clear halo around colonies, and the medium became yellow as previously described (Schau 1986) and then Gram' stain confirms to classify gram-positive, staphylococci arrangement, and catalase-positive. The typical colony and morphology of *Staphylococcus* spp. were found in all 16 samples (Table 2). Moreover, *Corynebacterium* spp., was detected with colony appearance on non-selective media TSA that was a small colony with circular, convex, shiny, white to the gray colony, and bacterial morphology was gram-positive, small, pleomorphic, non-sporing, catalase-positive, and appeared as club-shaped, V-in Y-shaped arrangements, or in clumps that resemble Chinese letters. This suggests that *Corynebacterium* spp. (Kasper & Fauci 2013; Bernard & Funke 2015), serves to indicate that *Corynebacterium* spp. was found in all 16 samples (Table 2). These results confirmed that both *Staphylococcus* spp. and *Corynebacterium* spp. were dominant in human armpit in both males and females, which is consistent with previous reports (Leaden et al., 1981; Troccaz et al., 2009; Yamazaki et al., 2010; Capone et al., 2011; James et al., 2013; Urban et al., 2016; Ågren et al., 2020). However, the specific confirmation of bacterial species should be required, especially using molecular biology techniques.

Consequently, the application of roll-on deodorants with *R. nasutus* extract could decrease armpit microbiomes of *Staphylococcus* spp. and *Corynebacterium* spp. thereby generating fatty acid witch induces pH increase providing optimal microenvironment for other microbial growth. The reduction of the two dominant odor causing bacteria, *Staphylococcus* spp. and *Corynebacterium* spp. odor causing bacteria would reduce armpit odor. Furthermore, the efficacy of plant extracts show that they are suitable to be used as a substitute for synthetic chemicals that cause irritation.

Table 2 Bacterial identification

Test	Bacteria	
	Stapphylococcus spp.	*Corenybacterium spp.*
Media	Baird-Parker agar (BPA)	Trypticase soy agar (TSA)
Colony	Grey-black, opaque halo, clear halo develops around colonies	White to gray, small, circular, convex, shiny
Gram' stain	Gram+	Gram+
Light microscopy	100X	100X
Morphology	Coccus shape, staphylococci arrangement, non-sporing	Bacilli shape, club-shaped, V-in Y-shaped arrange-ments, or in clumps, non-sporing
Catalase	+	+

Satisfaction assessment

Based on Fig. 2, the satisfaction assessment results of the volunteers after using the roll-on deodorant showed that the subjects were more satisfied with RDEOF-2 than RDEOF-1 with a score of reduction of body odor = (3.50, 3.25), absorption = (3.31, 2.88), color = (3.31, 3.25), spreading = (3.25, 3.06), product texture = (3.12, 3.06), smell = (2.81,2.69), and overall satisfied = (3.13, 3.06) respectively, while satisfaction after using the product = (3.00, 3.00) and viscosity = (3.13, 3.13) had the same level of satisfaction. On the other hand, regarding skin mildness = (3.50, 3.44), the subjects were more satisfied with RDEOF-1 than RDEOF-2. Certainly, the effects of deodorant products containing *R. nasutus* extract are indicating a decreased growth of bacteria that cause armpit odor. Therefore, the development of the deodorant product with natural plant extracts is warranted.

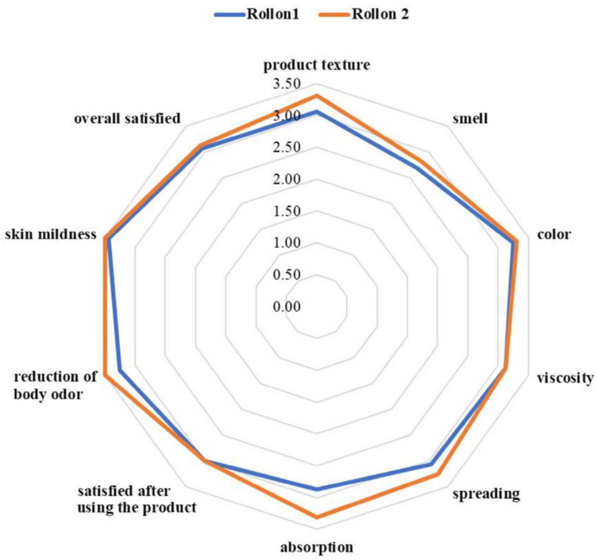

Fig. 2 Evaluation of RDEOF-1 (Roll-on deodorant formula-1) and RDEOF-2 (Roll-on deodorant formula-2) products by volunteer (n = 16) in satisfaction and product characteristics

Conclusion

We have confirmed that the two bacterial genera including *Staphylococcus* spp. and *Corenybacterium* spp. exhibit dominance in human armpit and causes axillary malodor. Using a roll-on deodorant containing *Rhinacanthus nasutus* (L.) extract could decrease armpit bacteria in order to reduce armpit odor. Satisfaction assessment results of the volunteers after using the roll-on deodorant showed that the subjects were more satisfied with RDEOF-2 than RDEOF-1.

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