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Short Communication

Topical Probiotics for Women's Urogenital Health: Selection of an Oil-based Carrier

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Abstract

Vaginal care products are widely used by women to relieve discomfort such as pain, itching and malodour, all of which are commonly caused by conditions resulting from microbiota dysbiosis. Previous studies showed that probiotic strains *Lacticaseibacillus* (formerly *Lactobacillus*) *rhamnosus* GR-1 (LGR-1) and *Limosilactobacillus* (formerly *Lactobacillus) reuteri RC-14 (LRC-14)*, can aid in restoring homeostasis in the vaginal microbiome when taken orally. A topical product containing these strains could be of value for reducing malodour and improving quality of life. However, the formulation of such a product is a challenge, given that its ingredients must maintain shelf-life viability by excluding moisture. Here, we tested petroleum jelly, mineral oil, coconut oil, and olive oil for how well they maintained the viability of freeze-dried probiotic strains over a six-month timeframe. None of the oils caused excessive loss of bacterial viability, with petroleum jelly and coconut oil showing the most promise. Based on existing knowledge of these oils on the female genitalia, coconut oil and petroleum jelly could be suitable probiotic carriers for clinical testing.

Keywords
Probiotics; vaginal health; urogenital health; personal care products

1. Introduction

Globally, feminine hygiene product revenue amounts to over 38 billion dollars, with a growth of 3.24% expected annually [1]. These products include washes, wipes, creams and sprays intended to clean, soothe, and treat the vaginal area. They are marketed for daily use or to relieve issues such as malodour. Although these products are intended to maintain vaginal comfort, many of them may induce adverse effects and disrupt the vaginal microbiota, inducing a state of dysbiosis [2-4] that can predispose women to bacterial vaginosis (BV), urinary tract infection (UTI), pregnancy complications [5], and sexually transmitted diseases [6], as well as have a negative emotional impact on wellbeing [7]. Therefore, new topical over-the-counter (OTC) therapies could be beneficial if they relieve the cause of aberrant symptoms and signs, and if they help maintain and restore vaginal homeostasis.

Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [8], have shown potential to improve female urogenital health. *Lactobacillus* species are dominant in the vaginal microbiome and have properties that contribute to health, such as lactic acid production [9] and various mechanisms that compete with pathogens [10]. *Lacticaseibacillus* (formerly *Lactobacillus*) *rhamnosus* GR-1 (LGR-1), taken orally, aids to maintain vaginal health and is the most studied probiotic strain for vaginal health [11]. Genomic analysis revealed that it is well adapted to the vaginal environment, specifically, due to a unique exopolysaccharide production cluster and its ability to metabolize lactose and maltose, as well as having increased resistance to oxidative stress [11].

Another strain of interest, *Limosilactobacillus* (formerly *Lactobacillus*) *reuteri* RC-14 (LRC-14) [12], in combination with LGR-1 can reduce infection recurrence [13-15]. This combination of strains also has antifungal activity against common uropathogenic yeast *Candida albicans* [16], and reduces the symptoms of vulvovaginal candidiasis (i.e. discharge, itching, and dysuria), when used in conjunction with an antifungal agent [17]. Furthermore, the symbiotic relationship between strains LGR-1 and LRC-14 can aid in the recovery from dysbiosis, replenishing indigenous species such as *Lactobacillus crispatus* and *Lactobacillus iners*, which are present in high abundance in a healthy vagina [13].

The aim of the present study was to test the viability of a commercial blend of LGR-1 and LRC-14 over a period of 6 months in olive oil, mineral oil, coconut oil, and petroleum jelly, as a means of developing a topical application of strains to counter pathogens and malodour. Current cream and oil-based products are invariably not probiotic by definition, nor do they guarantee that their ingredients can retain the viability of the bacterial contents [18]. The use of preservatives with bactericidal activity is ill-advised unless the compounds have proven safe for use in humans. However, this creates the problem of an increased risk of contaminants being in products. The use of oil-based compounds has the advantage of reduced (or null) water content making them less prone to contamination [19].

The oils used in the present study can be categorized as plant or petrolatum-based. The former includes olive oil and coconut oil, commonly used in cosmetics due to their low cost and moisturizing

properties. Both oils are composed of 95% triglycerides and have been shown to act as an emollient and to improve skin barrier function [19, 20]. Petroleum jelly and mineral oil are petrolatum-based and have a long-standing history of use in dermatology, dating back to the 1800s [21]. These oils are not absorbed into the skin, have a reduced allergenic profile, can act as an occlusive that reduces moisture loss [22, 23], and are highly stable [24, 25].

2. Materials and Methods

2.1 Oils

Petroleum jelly (Vaseline, Walmart), mineral oil (Life, Shopper's Drug Mart), coconut oil (Nutiva, Walmart), and olive oil (Gallo, Walmart), were purchased from local stores and kept sealed and not exposed to contamination throughout the duration of the experiment.

2.2 Microorganisms

Capsules from a commercial probiotic, containing 5 billion colony forming units (CFUs) of a blend of freeze-dried LRC-14 and LGR-1, were used in this study. Contents consisted of 1 g of bacteria-containing powder along with the following excipients: glucose anhydrate, microcrystalline cellulose, potato starch, magnesium stereate, gelatin, titanium dioxide, and milk.

2.3 Immersion of Bacteria in the Oil Carriers

Capsules were opened and their contents added to 2 mL tubes with 500 µL of each oil, and vortexed for 3 minutes. The control consisted of powder only. Petroleum jelly was melted by incubation in a heating block for 3 minutes prior to vortexing. Five replicates were used per oil per time point. Tubes were kept in the dark at a temperature of 20°C ± 2 and at a relative humidity of 50% ± 5.

2.4 Bacterial Extraction

The contents of the oils were extracted by adding 500 µL of sterile phosphate buffered saline (PBS, pH 7.4), vortexed for 5 minutes, and centrifuged at 5000 *g* for 15 minutes. The PBS was heated to 40°C before being added to the samples with petroleum jelly.

After centrifugation, two layers were formed: PBS with the precipitated bacteria at the bottom, and the oil at the top. Coconut, olive, and mineral oils were removed with a Pasteur pipette, and petroleum jelly with a sterile scoopula. Then, tubes were vortexed for 1 minute to thoroughly resuspend the contents.

2.5 Bacterial Quantification

Measurements of bacterial viability were made at 0, 1, 2, 3, 4, 5, and 6 months. Serial dilutions and CFU enumeration were performed using the drop plate method, which involved inoculating 12 mL of De Man, Rogosa, and Sharpe (MRS) [26] agar plates with rows of 5 µL drops of PBS containing bacteria using a multichannel pipette. Each row was a miniature serial dilution with each drop down the row having a dilution factor increased 10-fold. The agar plate was incubated anaerobically for

24 h at 37°C, and the number of colonies was determined by counting the row corresponding to the 10^8 dilution. The strains were not differentiated on the culture plates.

2.6 Statistical Analysis

The proportion of viable bacteria remaining in comparison to time 0 was calculated for each treatment at each time point. Statistical analysis was carried out in RStudio V1.2.1335, using the 'emmeans' package V1.6.0 [27] factorial two-way analysis of variance (ANOVA) with the Dunnett method post-hoc for multiple comparisons was used to calculate statistical significance. Data were plotted using 'ggplot2' [28] (Figure 1).

3. Results

3.1 Survival of Freeze-dried Probiotic Strains Stored in Different Oil Carriers

The percentage of bacterial survival is shown in Figure 1. Regardless of the treatment, all samples decreased in viability across time points, including the control, which consisted of powdered bacteria only. After 6 months, all samples had an average reduction in viability of 59.3%. Differences shown in Figure 1 are in comparison to the control at each specific time point.

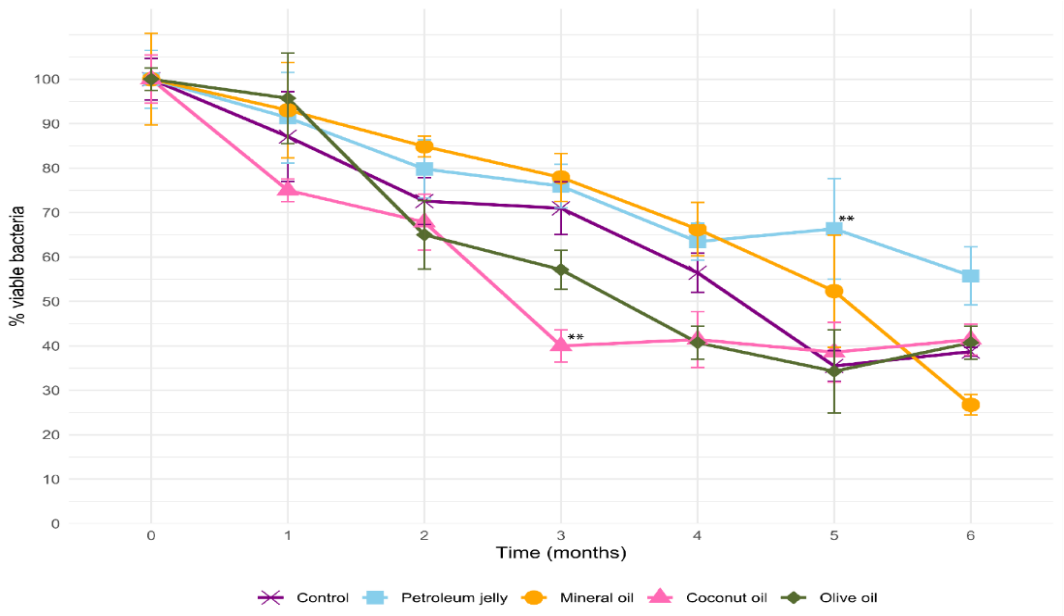

Figure 1 Effect of different oils on the viability of freeze-dried probiotic strains. The figure shows the proportion of CFUs remaining at time 0 and after months 1-6. One gram of a commercial blend of LGR-1 and LRC-14 was immersed in 500 µL of petroleum jelly (blue line, squared symbols), mineral oil (orange line, circular symbols), coconut oil (pink line, triangular symbols), or olive oil (green line, diamond symbols). The control consisted of freeze-dried bacteria and excipients only. Five independent experiments were carried out at every time point per treatment. The 100% represents 2 billion live organisms. Statistical significance was calculated with a factorial two-way analysis of variance (ANOVA) with the Dunnett method post-hoc for multiple comparisons (**p≤0.001). Differences shown compare each treatment against the control.

4. Discussion

The present study showed that several oils approved for vaginal use can be used to retain urogenital probiotic strain viability over six months. The use of personal care products either externally or intravaginally is common practice around the world [29-31]. These include vaginal washes, lubricants, and wipes ostensibly to relieve itching, dryness, malodour, or burning sensations, as well as to improve their sexual lives. Of critical importance in using local applications, in addition to safety, is that strains are selected for appropriate properties, and they can survive in the delivery vehicle. Unfortunately, some locally applied products can increase the risk of vaginal dysbiosis and subsequent BV or UTI [4, 31, 32].

There is generally a loss of bacterial viability within freeze-dried preparations. The product label states a guarantee that at least 1 billion of CFU would remain viable at the end of the shelf life of the product (approximately three years after manufacture). The viability of two probiotic strains was not reduced in mineral and olive oil compared to controls, indicating the oils were not antimicrobial. Of note, the powder used for these studies was from capsules which also provide protection from viability loss [33].

Both coconut oil and petroleum jelly are solid at room temperature, but they behaved differently. The strains incubated in coconut oil had decreased viability by almost 20% more after three months, yet no further loss occurred. The explanation is not known but suggests an equilibrium is reached perhaps after adaptation to the oil's properties. The viable count for strains incubated in petroleum jelly was higher at five compared to four months. We suspect was within experimental error despite the statistical significance, since the monthly trend followed a similar pattern between months one to four then five to six.

The nature of the coconut oil and petroleum jelly provided a relatively even dispersion of the bacterial powder. This is a desired attribute in this type of product, to ensure that every application contains the probiotic organisms. Therefore, if approximately 5mL was dispensed from a tube, at six months, it would still be expected to deliver one billion live organisms.

The knowledge of the impact of these oils in the vaginal microbiome is sparse. External use of mineral oil is common (i.e. perianally or on the vulval area) [34, 35], and despite it being linked to adverse reactions [29] and *Candida* colonization [31], it is a mainstay ingredient of medical pomades intended for vaginal use [36].

In considering the use of petroleum jelly to deliver probiotic bacteria, it should be noted that when applied intravaginally it is associated with a lower prevalence of *Lactobacillus* species and an increase in the abundance of BV-associated morphotypes [30, 31]. Yet, similarly to mineral oil, external use of petroleum jelly is generally considered safe and is common in clinical practice, either perianally [35], or on the skin of vulval vestibule to treat symptoms associated to dermatological inflammatory conditions of the vulva [34]. The inclusion of *Lactobacillus* strains antagonistic to BV organisms, could prove to counter these negative attributes of the jelly in the vagina. Better still, if the probiotic strains in petroleum jelly were only applied to the outer urogenital skin, this could reduce further the risk of BV organisms propagating.

Olive oil has antimicrobial, antioxidant, and anti-inflammatory activities, mostly due to its high content of phenolic compounds. However, little is known on its impact on the microbiota [37]. Its high content of oleic acid could harm the skin barrier and be an irritant, thereby damaging the native microbiota, which could in turn allow pathological organisms to colonize and cause inflammation

[20]. However, olive oil has been successfully used intravaginally in a clinical setting to relieve breast cancer patients of dyspareunia (genital pain caused by intercourse) and it can also inhibit *Candida* species [38].

Although coconut oil was the only tested oil to show a significant decrease in viability, it is also the one with most potential for vaginal health and, as mentioned previously, its ability to remain solid at room temperature allows for a better dispersion of freeze-dried bacteria. Coconut oil contains monolaurin, an antimicrobial monoglyceride formed from lauric acid, which is a short fatty acid that can disrupt the membranes of microbial organisms; it is particularly efficient at inhibiting common skin pathogens such as *Propionibacterium acne*s and *Staphylococcus aureus.* However, this antimicrobial effect could be the reason why there was a decrease in the viability of the probiotic strains at three months. This could pose challenges for product distribution, perhaps requiring refrigeration. Coconut oil has been shown to enrich commensals and decrease the expression of the pathogenesis pathway of fungi found on the scalp [39]. In addition, it is highly effective in reducing *Candida albicans* [40] and inhibiting the production of exotoxins by vaginal pathogens [41]. When applied vaginally to rhesus macaques, coconut oil did not affect the compositions of the vaginal microbiota [42].

A previous study has shown that application of LGR-1 to the vagina can stimulate antimicrobial peptides [42], making it a good choice for this type of application. An advantage of applying live bacteria rather than compounds such as lactic acid [43] is that the probiotic strains can adapt to the environment and produce other substances important to health maintenance.

It is also clear that packaging will play a major role for this type of product, both for enhancing viability, as well as to guarantee it is designed to be used within the ideal shelf-life time. Additionally, a product that must be kept in refrigeration would be an excellent approach to extend the utility life of the product while avoiding the use of additional ingredients (e.g., preservatives) that could affect the bacteria in the product as well as the urogenital microbiota of the consumer. Preparations formulated by apothecaries could be made by adding freeze-dried bacteria to the carrier with a defined short-term shelf life, thereby avoiding distribution issues with a pre-made cream.

Of note, all the tested oils retained the viability of probiotics in a comparable manner as the control, suggesting that they can also be used when formulating topical probiotic products with other stains that target different organs, such as the skin.

In summary, the present study suggests that petroleum jelly and coconut oil are good candidates as vehicles for the delivery of topical probiotics to the external female genitalia. Future studies could assess the impact on the vaginal microbiota and metabolic read-out as well as the effects on epithelial cells. More specifically, clinical trials with large sample sizes must be performed where participants apply the ingredients of interest in the vaginal area for at least 6 months, during which swabs would be taken weekly in order to take hormonal changes into account. Metabolomic and transcriptomic analyses should then be performed to better understand the microbial dynamics and the impact of the compound of interest.

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Author Contributions

SPB and BL wrote the main draft. GR and SJ proof-read the manuscript and provided feedback. GR conceptualised the project. SPB managed the project. SPB, SJ, and BL carried out the experiments. SPB performed the data analysis and visualization.

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Competing Interests

The authors have declared that no competing interests exist.

References

1. Statista Research Department. Feminine hygiene market-Statistics & Facts [Internet]. New York: Statista; 2020 [cited date 2021 May 10th]. Available from: https://www.statista.com/topics/4889/feminine-hygiene-market/.
2. Fashemi B, Delaney ML, Onderdonk AB, Fichorova RN. Effects of feminine hygiene products on the vaginal mucosal biome. Microb Ecol Health Dis. 2013; 24: 19703.
3. Jenkins A, Money D, O'Doherty KC. Is the vaginal cleansing product industry causing harm to women? Expert Rev Anti Infect Ther. 2021; 19: 267-269.
4. Chen Y, Bruning E, Rubino J, Eder SE. Role of female intimate hygiene in vulvovaginal health: Global hygiene practices and product usage. Womens Health. 2017; 13: 58-67.
5. Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin, DH, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. N Engl J Med. 1995; 333: 1737-1742.
6. Martin Jr HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis. 1999; 180: 1863-1868.
7. Bilardi JE, Walker S, Temple-Smith M, McNair R, Mooney-Somers J, Bellhouse C, et al. The burden of bacterial vaginosis: Women's experience of the physical, emotional, sexual and social impact of living with recurrent bacterial vaginosis. PLoS One. 2013; 8: e74378.
8. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11: 506-514.
9. Tachedjian G, Aldunate M, Bradshaw CS, Cone RA. The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. Res Microbiol. 2017; 168: 782-798.
10. Reid G, Younes JA, van der Mei HC, Gloor GB, Knight R, Busscher HJ. Microbiota restoration: Natural and supplemented recovery of human microbial communities. Nat Rev Microbiol. 2010; 9: 27-38.

11. Petrova MI, Reid G, Ter Haar JA. *Lacticaseibacillus rhamnosus* GR-1, aka *Lactobacillus rhamnosus* GR-1: Past and future perspectives. Trends Microbiol. 2021; 29: 747-761.
12. Zheng J, Wittouck S, Salvetti E, Franz CM, Harris H, Mattarelli P, et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol. 2020; 70: 2782-2858.
13. Macklaim JM, Clemente JC, Knight R, Gloor GB, Reid G. Changes in vaginal microbiota following antimicrobial and probiotic therapy. Microb Ecol Health Dis. 2015; 26: 27799.
14. Reid G. The development of probiotics for women's health. Can J Microbiol. 2017; 63: 269-277.
15. Vujic G, Knez AJ, Stefanovic VD, Vrbanovic VK. Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: A double-blind, randomized, placebo-controlled study. Eur J Obstet Gynecol Reprod Biol. 2013; 168: 75-79.
16. Martinez RCR, Seney SL, Summers KL, Nomizo A, De Martinis ECP, Reid G. Effect of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on the ability of *Candida albicans* to infect cells and induce inflammation. Microbiol Immunol. 2009; 53: 487-495.
17. Martinez RCR, Franceschini SA, Patta MC, Quintana SM, Candido RC, Ferreira JC, et al. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. Lett Appl Microbiol. 2009; 48: 269-274.
18. Puebla-Barragan S, Reid G. Probiotics in cosmetic and personal care products: Trends and challenges. Molecules. 2021; 26: 1249.
19. Sarkar R, Podder I, Gokhale N, Jagadeesan S, Garg VK. Use of vegetable oils in dermatology: An overview. Int J Dermatol. 2017; 56: 1080-1086.
20. Vaughn AR, Clark AK, Sivamani RK, Shi VY. Natural oils for skin-barrier repair: Ancient compounds now backed by modern science. Am J Clin Dermatol. 2018; 19: 103-117.
21. Draelos ZD. The science behind skin care: Moisturizers. J Cosmet Dermatol. 2018; 17: 138-144.
22. Patzelt A, Lademann J, Richter H, Darvin ME, Schanzer S, Thiede G, et al. In vivo investigations on the penetration of various oils and their influence on the skin barrier. Skin Res Technol. 2012; 18: 364-369.
23. Chuberre B, Araviiskaia E, Bieber T, Barbaud A. Mineral oils and waxes in cosmetics: An overview mainly based on the current European regulations and the safety profile of these compounds. J Eur Acad Dermatology Venereol. 2019; 33: 5-14.
24. Nash JF, Gettings SD, Diembeck W, Chudowski M, Kraus AL. A toxicological review of topical exposure to white mineral oils. Food Chem Toxicol. 1996; 34: 213-225.
25. Rawlings AV, Lombard KJ. A review on the extensive skin benefits of mineral oil. Int J Cosmet Sci. 2012; 34: 511-518.
26. De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. J Appl Bacteriol. 1960; 23: 130-135.
27. Lenth R. Emmeans: Estimated marginal means, aka least-squares means [Internet]. R package version 1.7.0. 2021. CRAN. Available from: https://CRAN.R-project.org/package=emmeans.
28. Wickham H. Ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag; 2016.
29. Crann SE, Cunningham S, Albert A, Money DM, O'Doherty KC. Vaginal health and hygiene practices and product use in Canada: A national cross-sectional survey. BMC Womens Health. 2018; 18: 52.

30. Hassan WM, Lavreys L, Chohan V, Richardson BA, Mandaliya K, Ndinya-Achola JO, et al. Associations between intravaginal practices and bacterial vaginosis in Kenyan female sex workers without symptoms of vaginal infections. Sex Transm Dis. 2007; 34: 384-388.
31. Brown JM, Hess KL, Brown S, Murphy C, Waldman AL, Hezareh M. Intravaginal practices and risk of bacterial vaginosis and candidiasis infection among a cohort of women in the United States. Obstet Gynecol. 2013; 121: 773-780.
32. Brown JM, Poirot E, Hess KL, Brown S, Vertucci M, Hezareh M. Motivations for intravaginal product use among a cohort of women in Los Angeles. PLoS One. 2016; 11: e0151378.
33. Wilcox H, Carr C, Seney S, Reid G, Burton JP. Expired probiotics: What is really in your cabinet? FEMS Microbes. 2020; 1: xtaa007.
34. Thorstensen KA, Birenbaum DL. Recognition and management of vulvar dermatologic conditions: Lichen sclerosus, lichen planus, and lichen simplex chronicus. J Midwifery Womens Health. 2012; 57: 260-275.
35. Araújo NM, Oliveira SM. The use of liquid petroleum jelly in the prevention of perineal lacerations during birth. Rev Lat Am Enfermagem. 2008; 16: 375-381.
36. Tirri B. Antimicrobial topical agents used in the vagina. In: Topical Applications and the Mucosa. Basel: Karger; 2011. pp.36-47.
37. Cicerale S, Lucas L, Keast R. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr Opin Biotechnol. 2012; 23: 129-135.
38. Alwan AJ, Alwan SJ. Comparing the effect of some plant extracts and commercial washes against some isolated microorganisms from vagina of pregnant women. Ann Trop Med Public Health. 2019; 22: 65-75.
39. Saxena R, Mittal P, Clavaud C, Dhakan DB, Roy N, Breton L, et al. Longitudinal study of the scalp microbiome suggests coconut oil to enrich healthy scalp commensals. Sci Rep. 2021; 11: 7220.
40. Ogbolu DO, Oni AA, Daini OA, Oloko AP. In vitro antimicrobial properties of coconut oil on Candida species in Ibadan, Nigeria. J Med Food. 2007; 10: 384-387.
41. Schlievert PM, Strandberg KL, Brosnahan AJ, Peterson ML, Pambuccian SE, Nephew KR, et al. Glycerol monolaurate does not alter rhesus macaque (*Macaca mulatta*) vaginal lactobacilli and is safe for chronic use. Antimicrob Agents Chemother. 2008; 52: 4448-4454.
42. Kirjavainen PV, Laine RM, Carter DE, Hammond JA, Reid G. Expression of anti-microbial defense factors in vaginal mucosa following exposure to *Lactobacillus rhamnosus* GR-1. Int J Probiotics Prebiotics. 2008; 3: 99-106.
43. Bruning E, Chen Y, McCue KA, Rubino JR, Wilkinson JE, Brown ADG. A 28 day clinical assessment of a lactic acid-containing antimicrobial intimate gel wash formulation on skin tolerance and impact on the vulvar microbiome. Antibiotics. 2020; 9: 55.

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