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Formulation and evaluation of intimate wash containing manuka oil and salix bark extract

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ABSTRACT: Most of the feminine hygiene products sold in the market contain heavy chemicals that damage the mucosa. The well-designed intim formulation cleans without damage, supports the flora, protects against dryness, and maintenances of balanced microflora. For this purpose, we designed formulations made with natural raw materials such as manuka oil and *Salix alba* bark extract and investigated their irritation potential and effectiveness. A healthy vagina is dominated by Lactobacillus that produces lactic acid, resulting in a characteristic acidic environment (pH 3–4), as it correlates with vaginal health by inhibiting the growth of bacteria and may also play a part in local immune defense. This new manuka oil- salix bark extract containing wash formulation was specifically formulated to achieve a formulation pH of 4.6-4.8 to be compatible with the normal skin pH range and to help maintain vulvovaginal skin homeostasis and provide protection against harmful bacteria. The formulation we designed was used by volunteers for 4 weeks and was found suitable in terms of gentle clean, refreshing feeling, shooting the skin, smell and irritation properties. None of the volunteers have experienced any side effects during product application. Our study findings further support other studies that have found that women who use intim wash attitudes and perceptions about hygiene.

KEYWORDS: Feminine wash; manuka oil; salix bark extract; female intimate hygiene; vulvovaginal.

1. INTRODUCTION

Women often use a variety of feminine hygiene products as part of their daily cleaning routine; including soaps, body washes, foam, premoistened wipes, powders, and deodorant sprays.

However, there is little published medical literature on the external vulva and how personal hygiene practices may affect it. In many cases, proper intimate feminine hygiene can help prevent or relieve the troublesome symptoms of itching and abnormal vaginal discharge and improve overall well-being [1].

Supported by international guidelines, daily gentle cleansing of the vulva is an important aspect of feminine hygiene and general intimate health. The Royal College of Obstetricians and Gynaecologists (RCOG) and the Middle East and Central Asia (MECA) performed extensive literature searches to develop evidence-based guidelines intended. Both guidelines suggest daily vulva cleansing with a mild hypoallergenic liquid wash in pH 4.2 to 5.6 [1,2].

The vulva is the first line of defense to protect the genital tract from infection. Contaminants often collect in the vulvar folds, and increased moisture, sweating, menses, and hormonal fluctuations influence vulvar microbial growth and species balance, potentially resulting in odor and vulvovaginal infection., The genital skin is more susceptible to topical agents than forearm skin. Because vulvar skin is covered by large hair follicles and happened intensive hydration, permeability is increased [3-8].

The pH of the vulvar skin is between 4.7 (skin) [9] and 3.5 (vagina) [10] and it has been reported that it can vary between 3.8 and 4.2 during the menstrual cycle [11].

Most of the feminine hygiene products sold in the market contain heavy chemicals that damage the mucosa [1]. The well-designed intim formulation cleans without damage, supports the flora, protects against dryness, and maintenances of balanced microflora. For this purpose, we designed formulations made with natural raw materials such as manuka oil and *Salix alba* bark extract and investigated their irritation potential and effectiveness.

Mānuka oil (CAS 219828- 87-2) is a volatile essential oil derived from the foliage, bark and seeds of *Leptospermum scoparium* plants of the Myrtaceae family of trees and shrubs. Mānuka grows abundantly throughout New Zealand and has been a part of traditional Maori medicine for a variety of applications like

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antibacterial, antifungal, anti-parasitic/insecticidal, anti-inflammatory, antiviral and spasmolytic activity from leptospermone, iso-leptospermone ve flavesone [12].

Salix bark extract has been used for thousands of years as an anti-inflammatory, antipyretic, and analgesic. Salix bark extracts can have anti-inflammatory and antiviral properties [13,14]. The proanthocyanidin fractions of willow bark extracts have also been reported to have antiviral and antibacterial activities [15]. Overall, proanthocyanidins have been characterized as antioxidant, antibacterial, antitumor, anticancer, neuroprotective, hypoglycemic, and lipid-lowering activities [16]. A study observed that while the number of *Enterobacteriaceae* and *Escherichia coli* decreased, the beneficial lactobacilli increased thanks to the bark extract of *Salix alba* on the cecal microbial population in broilers [17]. For this purpose the extract can be use to inhibit of pathogen microorganims without affecting the beneficial flora that already exist for prebiotic action in genital hygiene [18].

2. RESULTS

2.1. Materials

The physicochemical and sensorial characterization parameters of the formulation are reported in Table 1. A gel-like, fresh-smelling, clear and colorless formulation was obtained. The pH of the formulation was between 4.6- 4.8. The density was found 1.02 ± 0.2 g/mL. The viscosity was found 32 ± 0.7 P. The physicochemical and sensorial parameters of the formulation were appropriate for the dermal application.

Table 1. Physicochemical and sensorial characterization parameters of the formulation are reported.

Physicochemical Parameters	Sensorial Parameters
pH range: 4.6-4.8	Appearance: Gel
Density (g/ml): 1.02± 0.2	Odor: Fresh
Viscosity (P): 32± 0.7	Color: Clear

2.2. Microbial assays

All results were given in Table 2. No microbial growth was observed and formulation suitable for clinical evaluations.

Table 2. Microbial assay results are reported.

Test Microorganisms	Microbiological Parameters
Total *Bacteria*	<100 CFU*/mL
Yeast and *Mould*	None
Escherichia coli	None
Staphylococcus aureus	None
Pseudomonas aeruginosa	None
Candida albicans	None

* CFU: colony-forming unit

2.3. Questionnaire results

The survey was completed by 20 respondents. The median age was 42.50 (IQ range 21–64) years old. Volunteers declared that the formulation is efficient in (Table 3):

- gently cleanses the intimate areas (at 90%),
- provides a refreshing feeling (at 90%),
- doesn't irritate intimate areas (at 80%),
- soothes the skin of intimate areas (at 85%),
- pleasant smell (at 90%).

None of the volunteers have experienced any side effects during product application (Figure 1).

Table 3. Questionnaire results are reported.

CLAIM	ANSWER	PEOPLE AMOUNT	%	POSITIVE ANSWERS CONFIRMATION DECLARATIONS (%) OF
GENTLY CLEANSES THE INTIMATE AREAS	definitely no	0	0,00	90
	no	0	0,00	
	neutral	2	10,00	
	yes	15	75,00	
	definitely yes	3	15,00	
PROVIDES A REFRESHING FEELING	definitely no	0	0,00	90
	no	0	0,00	
	neutral	2	10,00	
	yes	15	75,00	
	definitely yes	3	15,00	
DO YOU AGREE THAT PRODUCT DOESN'T IRRITATE INTIMATE AREAS	definitely no	1	5,00	80
	no	1	5,00	
	neutral	2	10,00	
	yes	15	75,00	
	definitely yes	1	5,00	
SOOTHES THE SKIN OF INTIMATE AREAS	definitely no	0	0,00	85
	no	0	0,00	
	neutral	3	15,00	
	yes	15	75,00	
	definitely yes	2	10,00	
PLEASANT SMELL	definitely no	0	0,00	90
	no	1	5,00	
	neutral	1	5,00	
	yes	16	80,00	
	definitely yes	2	10,00	

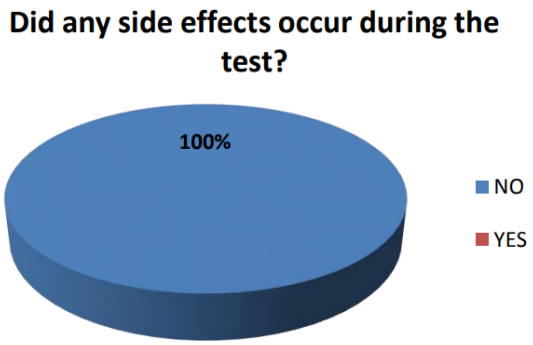

Figure 1. Side effects during product application are reported.

2.4. Interpretation of patch test

Reading the test and writing their results have been done in accordance with the recommendations of the International Contact Dermatitis Research Group (ICDRG) (Table 4, Figure 2). None of 15 people, who were exposed to patch testing showed positive reactions during the test reading (Table 5, Figure 3).

Table 4. The recommendations of the International Contact Dermatitis Research Group (ICDRG).

Record	Diagnosis	Interpretation
-	Negative reaction	No skin lesions
?	Doubtful reaction	Faint erythema only
+	Weak positive reaction	Palpable erythema, infiltration, possibly papules
++	Strong positive reaction	Erythema, infiltration, papules, vesicles
+++	Extreme positive reaction	Intense erythema, infiltration and coalescing vesicles, bullous or ulcerative reaction
IR	Irritant reaction of different types	Discrete patchy erythema without infiltration

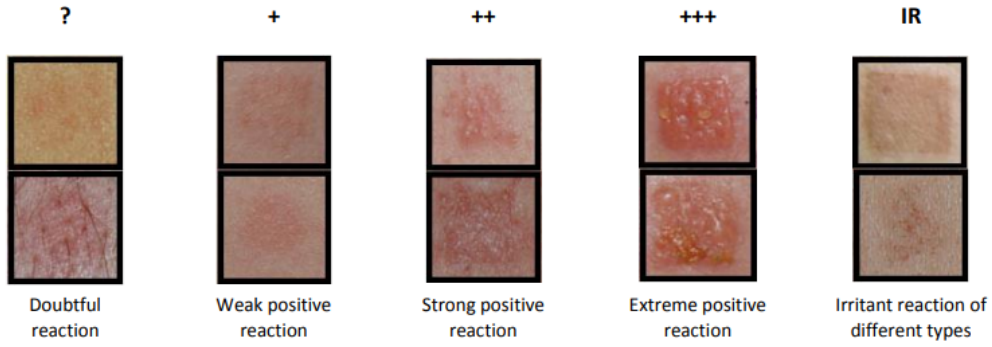

Figure 2. The recommendations of the International Contact Dermatitis Research Group (ICDRG).

3. DISCUSSION

Feminine hygiene is very important stage in women's health. Because some chemicals like surfactants may irritate the vulvovaginal skin, intimate wash formulations should be formulated and tested to balanced the pH and natural microflora of the genital area. Incorrect intimate product usage and hygiene practise can cause various vulvovaginal disorders [1].

A healthy vagina is dominated by *Lactobacillus* that produces lactic acid, resulting in a characteristic acidic environment (pH 3–4), as it correlates with vaginal health by inhibiting the growth of bacteria [10] and

may also play a part in local immune defense [19]. This new manuka oil- salix bark extract containing wash formulation was specifically formulated to achieve a formulation pH of 4.6-4.8 to be compatible with the normal skin pH range and help maintain vulvovaginal skin homeostasis and provide protection against harmful bacteria. This pH values consistent with the literature [9,10,11,20,21].

External feminine washes, formulated to an acidic pH that enhances skin homeostasis, are considered more appropriate than internal washes or douches and may be a useful adjunct therapy for women with vaginal infections or taking antibiotics [22,23].

This study is about investigating the effectiveness of feminene wash formulation (prepared by manuka oil and salix bark extract which are known to have antimicrobial, prebiotic anti-inflammatory properties) with a survey and the irritation potential with a patch test. Several new findings emerged from our study. Although the number of participants is limited, the low-sensitivity formulation was designed with the use of natural substances and did not cause any irritation despite it contains alkyl glucosides. Alkyl glucosides are non-ionic, biodegradable, surfactants and are synthesized from glucose and fatty alcohol linked together with a glucoside bond. Therefore, alkyl glucosides are used in personal care products and cosmetics [24]. Alkyl glucosides can cause allergic contact dermatitis in cosmetics. However, the mechanism by which these substances unexpectedly cause sensitization is not clear [25].

To our knowledge, no studies have examined feminine hygiene products containing salix bark and manuka oil. The formulation we designed was used by volunteers for 4 weeks and was found suitable in terms of gentle clean, refreshing feeling, shooting the skin, smell and irritation properties. Our study findings further support other studies that have found that women who use intim wash beliefs, attitudes, and perceptions about hygiene [26-28].

Table 5. The patch test results are reported.

No.	Identification number	Sex	Age	Test result			
				after 1-st application	after 2-nd application	after 3-rd application	after one week from 1-st application
1	09/08/19/D/9-1	F	19	**(-)**	**(-)**	**(-)**	**(-)**
2	09/08/19/D/9-2	F	56	**(-)**	**(-)**	**(-)**	**(-)**
3	09/08/19/D/9-3	F	55	**(-)**	**(-)**	**(-)**	**(-)**
4	09/08/19/D/9-4	F	46	**(-)**	**(-)**	**(-)**	**(-)**
5	09/08/19/D/9-5	F	37	**(-)**	**(-)**	**(-)**	**(-)**
6	09/08/19/D/9-6	F	31	**(-)**	**(-)**	**(-)**	**(-)**
7	09/08/19/D/9-7	F	56	**(-)**	**(-)**	**(-)**	**(-)**
8	09/08/19/D/9-8	F	22	**(-)**	**(-)**	**(-)**	**(-)**
9	09/08/19/D/9-9	F	22	**(-)**	**(-)**	**(-)**	**(-)**
10	09/08/19/D/9-10	F	57	**(-)**	**(-)**	**(-)**	**(-)**

11	09/08/19/D/9-11	F	26	**(-)**	**(-)**	**(-)**	**(-)**
12	09/08/19/D/9-12	F	26	**(-)**	**(-)**	**(-)**	**(-)**
13	09/08/19/D/9-13	F	27	**(-)**	**(-)**	**(-)**	**(-)**
14	09/08/19/D/9-14	M	34	**(-)**	**(-)**	**(-)**	**(-)**
15	09/08/19/D/9-15	F	26	**(-)**	**(-)**	**(-)**	**(-)**

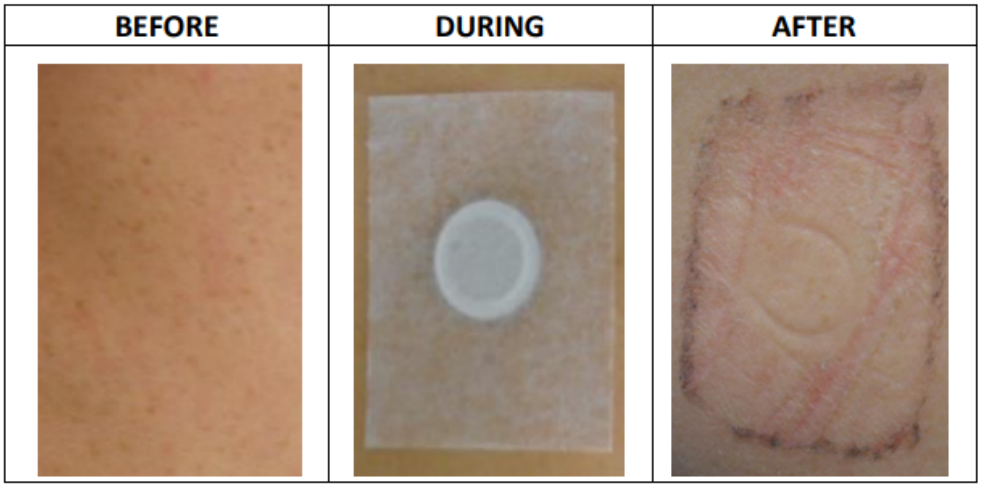

Figure 3. This is the pre-evaluation image of the patch test.

4. CONCLUSION

This is the first study to evaluate the effect of a feminine wash containing manuka oil and salix bark extract on the natural pH. The study showed that the developed formulation gently cleans, does not irritate and relaxes of the skin in the intimate areas. It also has a pleasant smell and refreshing feeling.

5. MATERIALS AND METHODS

5.1. Materials

The chemicals such as cocamidopropyl betaine, lauryl glucoside, disodium laureth sulfosuccinate, disodium cocoamphodiacetate, decyl glucoside, coco-glucoside, glyceryl oleate, parfum, methylpropanediol, polysorbate 80, Manuka oil (isoleptospermone, isoleptospermone, flavesone) *Salix alba* bark extract, sodium benzoate, glycerin, citric acid, PEG/PPG-120/10 trimethylolpropane trioleate, laureth-2, potassium sorbate and distilled water were used in the formulation. The mixture was prepared with the ingredients added in the specified proportions.

5.2. Characterization of formulation

The formulation was evaluated for their sensorial (appearance, odor, color) and physicochemical (pH viscosity density) parameters detected by a digital pH-meter (Mettler Toledo S 220, Switzerland), a

pycnometer (Mettler Toledo 30330857, Switzerland), and a viscometer (Brookfield RVDVII, Rheocalc V2.4, cone spindle no: 52, UK). The experiments were repeated five times at 25°C.

5.3. Microbial assays

The microbiological contamination of formulations was evaluated by validated tests methods TS EN ISO 22718, 22717, 21149, 16212, 18416 for cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli, Candida albicans* and total yeast and mold [29].

5.4. Patch test

Dermatological tests were performed in accordance with the Declaration of Helsinki and COLIPA Guidelinens for the Assessment of Human Skin Compatibility. Test has been conducted a group of 15 individuals using h-RIPT (Human Repeated Insult Patch Test) model. Reading the tests and results registration has been done in accordance with the recommendations of the International Contact Dermatitis Research Group (ICDRG). The report from application research number is 09/08/19/D/9. Informed consent was obtained from all participants.

14 women and 1 man, aged 19 – 56 years, with sensitive, problematic, dry skin were selected for the dermatological tests of the product. All of the probands selected for testing met the requirements for inclusion in the study, signed an agreement to participate in the study and were informed about: the purpose of the study, how it is carried out and what are the possible side effects. During the tests all the probands were under constant dermatological care.

A small amount of product (1% w/v suspension in distilled water) was applied to the patients forearm using patch test for 3 following days and then removed. Baseline readings were recorded half an hour after removal to let erythema from patches and tape (if any) to settle down. Additional readings were performed after one week for products that may show delayed reactions. Readings evaluation was done according to graphic scale which was consistent with generally accepted clinical dermatological scale (Table 4, Figure 2).

5.5. Questionnaire

Volunteers taking part in the study were selected on the bases of:
▪ Current Polish and European law,
▪ COLIPA Guidelines,
▪ Declaration of Helsinki (1964) (with later additions).

Volunteers received the product and the previously prepared questionnaire. Volunteers were informed about frequency and area of usage. The tests lasted for 4 weeks. All volunteers were obliged to:
▪ use product for 4 weeks,
▪ not use any products with similar function,
▪ urgently stop using product if any unwanted side effects occurred and immediately report to dermatologist or gynaecologist,
▪ observe and note down all the observation in questionnaire.
Tests were completed by all enrolled people.

5.6. Statistical Analysis

The raw data analysis was performed by using MS Excel Software.

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Author contributions: Concept – G.G.; Design – G.G.; Supervision – G.G.; Resources –G.G.; Materials – G.G.; Data Collection and/or Processing – G.G.; Analysis and/or Interpretation – G.G.; Literature Search – G.G.; Writing – G.G.; Critical Reviews – G.G.

Conflict of interest statement: The authors declared no conflict of interest.

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