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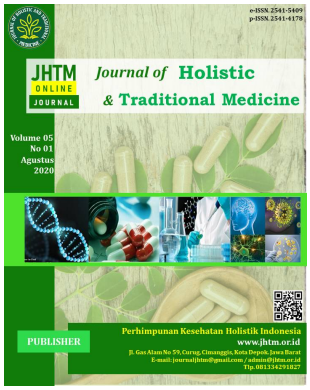

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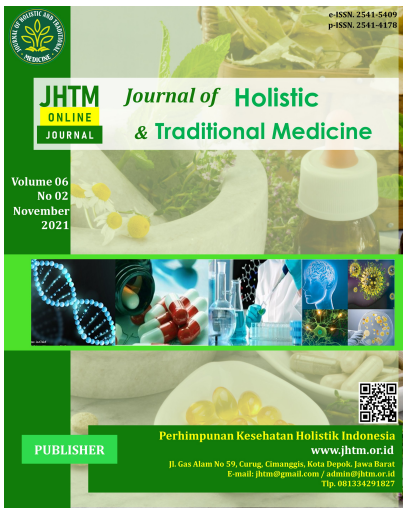

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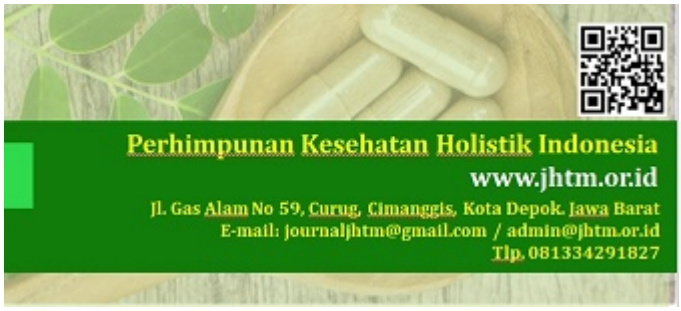

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FROM LABORATORY TO CLINICAL TREATMENT OF HYPERPIGMENTATION POST-INFLAMMATORY, ANTI-MICROBA AND BODY ODOR IN AXILLARY REGION WITH TISSUE INNOVATION COMPOSITED PIONIN, ALOE VERA, GLUTATHIONE, AND LAVENDER

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ABSTRACT

It is generally recognized that hyperpigmentation post-inflammatory and body odor issues can be distressing. This will also have an effect on the overall quality of life. As a result, we require a therapy that may address both post-inflammatory hyperpigmentation and body odor issues. The goal of this study was to see if the invention's combination had any antibacterial properties, as well as if it might lessen or fade the signs of hyperpigmentation post-inflammatory. It is believed that the antibacterial action of the current innovation would minimize patients' body odor. This composition is in accordance with the Regulation of the Head of the Drug and Food Control Agency of the Republic of Indonesia Number 12 of 2019 concerning Contaminants in Cosmetic. The axillary tissue cleansing formulation comprises pionin, aloe vera, glutathion, and lavender, all of which have been shown in the laboratory to prevent bacterial development. Clinically, however, the administration of this intervention was demonstrated to be capable of reducing or fading the symptoms of hyperpigmentation post-inflammatory and relieving body odor with very little adverse effects.

Keywords: *body odor; microorganism of skin; pionin, lavender; glutathione; hyperpigmentation post-inflammatory; axillary*

1. INTRODUCTION

One of the most prevalent dermatological problems in individuals with pigmented skin is post-inflammatory hyperpigmentation. After external damage, it causes an increase in melanin production or an aberrant distribution of this pigment in the epidermis and/or dermis. Prostaglandins, reactive oxygen species, thromboxanes, and leukotrienes are all recognized inflammatory mediators that activate melanocytes. Destruction of the basal cell layer in the dermis has also been documented using incontinentia pigmenti and melanophages. [1–3]

Although the exact prevalence of axillary hyperpigmentation in Latin-American ladies is unclear, it is a common dermatological issue. Histopathologically, it is distinguished by a rise in melanocytic activity and melanin deposits in both the epidermis and dermis, as well as an inflammatory infiltration dominated by mononuclear cells and macrophages. These data lend credence to the idea that axillary hyperpigmentation is a form of post-inflammatory hyperpigmentation, with the

precipitating causes being persistent irritation from hair removal, washing, tight clothing, or antiperspirant usage. The increased frequency of axillary hyperpigmentation among darker-skinned phototypes may be due to inherent characteristics in this group. These characteristics may include the existence of genetically labile melanocytes that are quickly killed by trauma and inflammation, as well as a more active response to inflammatory stimuli.[1,4–7]

On the other hand, one of the problems that are often experienced in the armpit is the problem of body odor. This is due to the accumulation of pathogenic bacteria. Bromhidrosis, also known as osmidrosis or malodorous sweating, is an unpleasant disease marked by foul body odor, particularly in the axillary, vaginal, and foot areas. All three kinds of sweat glands contribute to the development of this illness. Sweating excessively, followed by bacterial breakdown of sweat components, resulting in an unpleasant odor of perspiration. Apocrine bromhidrosis, which occurs after puberty as opposed to eccrine bromhidrosis, is the most prevalent type of this disease. Bacteria degrade apocrine sweat into a variety of volatile compounds, including ammonia and short chain fatty acids, such as (E)-3-methyl-2-hexenoic acid (E3M2H), a C7 branched and unsaturated acid. It is said to have a very strong, pungent odor. Natsch et al. discovered that odorous E3M2H, as well as its hydrated analogue, (R)/(S)-3-hydroxy-3-methylhexanoic acid ((R)/(S)-HMHA), are released from glutamine conjugates (present in axilla secretions) by a specific zinc-dependent N—acyl-glutamine aminoacylase (N-AGA) from commensal Corynebacterum species The most abundant has been observed to be HMHA (which has a rotten, cheesy odor). Another particle responsible for axillary malodor is the (S)-isomer of 3-methyl-3-sulfanylhexan-1-ol ((R)/(S)-MSH), which has an oniony, clary sage-like odor. Furthermore, eccrine bromhidrosis has been linked to a number of variables. Ingestion of specific foods such as garlic or onion, bacterial breakdown of keratin, metabolic problems, and hyperhidrosis are examples of these. [8–12]

It is well known that hyperpigmentation post inflammatory and body odor problems can make a person uncomfortable. This will also have an impact on the quality of human life. Therefore we need a treatment that can include the improvement of post-inflammatory hyperpigmentation and body odor problems. The present invention is an axillary cleansing formulation containing Pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06% distilled water up to 100%. has antimicrobial effects, brightens, refreshes and eliminates unpleasant armpit odors, especially in the armpits, this formula provides added value practically when traveling, when caring for sick people or when working overtime.

The purpose of this study was to laboratory test the antibacterial effect of the mixture of the invention, as well as its effect in lightening or fading the symptoms of post-inflammatory hyperpigmentation. It is hoped that the antibacterial effect of the present invention will reduce the body odor experienced by patients

2. METHOD AND MATERIAL

Laboratorium

The formulation of the cleanser solution was made by mixing pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06%, then added aquabides as a solvent. up to 100% concentration. The cleaning solution is mixed with tissue sheets measuring 18-54 cm x 16-48 cm which are processed in a machine. The output is body tissue that has been wrapped in a special container. The container states how to use it.

This research was carried out in an accredited laboratory, namely SIG.

This research was conducted on September 24, 2021 with the test sample number being 21J013SSTBS-A. The experiment was carried out with a test temperature of 24 degrees Celsius and the incubation temperature for the test bacteria. The test bacteria used in this study was Staphylococcus aureus (ATCC 6538). There are also other parameters that were tested in this study in the form of Yeast Mold Number, Total Plate Number, and Pseudomonas aeruginosa. The initial amount given for S. aureus test microbes was 50,000,000 CFU/mL, with contact times of 30 seconds and 60 seconds. Statistical analysis used in this study is descriptive statistical exposure.

Clinical Study:

This study is a retrospective cohort study in which a sample of women with post-inflammatory hyperpigmentation in the armpit area. The hyperpigmentantion occurs due to shaving the hair in the armpits. This study takes medical record data from January 2016 to January 2018. The minimum required sample size is 30 people. The inclusion criteria in this study were women aged 18 to 35 years. The exclusion criteria in this study were history of allergies with mixed formulation ingredients and incomplete medical records. The independent variable in this study was the use of tissue containing pionin, lavender, aloe vera, and glutathione. The dependent variable in this study was the improvement of post-inflammatory hyperpigmentation color as measured by The Taylor Hyperpigmentation Scale method. The Taylor Hyperpigmentation Scale is a visual novel scale that may be used to assess all skin types. This tool was created to give a low-cost and simple way for assessing hyperpigmentation and measuring progress in hyperpigmentation after therapy. The length of observation used was 8 weeks. The statistical analysis used in this study is a paired t-test statistical and Mc-Nemar Test analysis with an alternative test in the form of the Wilcoxon test (Alternatif test from paired t-test).

3. RESULTS

Testing of body cleansing tissue formulation containing pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06%,, the final results showed that the number of yeasts was < 10 colonies/g, the total plate number was < 10 colonies/g, Staphyloccus aureus was negative, Pseudomonas Aeuriginosa was negative, and Candida albicans was negative. This composition is in accordance with the Regulation of the Head of the Drug and Food Control Agency of the Republic of Indonesia Number 12 of 2019 concerning Contaminants in Cosmetics. (Table I)

Table I. Testing of body cleansing tissue formulation containing pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06%

No.	Parametric	Results	Baseline	Metric	Method
1	Yeast Mold Number	< 10	$\leq 10^3$	Colony/g	PP.16.18- Mikro/17-25/LABSKESDA
2	Total Plate Number	< 10	$\leq 10^3$	Colony/g	PP.16.18- Mikro/17-25/LABSKESDA
3	Staphylococcus aureus	Negatif	Negatif	Per 0,1 g	PP.16.18- Mikro/17-25/LABSKESDA

4	Pseudomonas aeruginosa	Negatif	Negatif	Per 0,1 g	PP.16.18- Mikro/17-25/LABSKESDA
5	Candida albicans	Negatif	Negatif	Per 0,1 g	PP.16.18- Mikro/17-25/LABSKESDA

Testing the effectiveness or antimicrobial effect of the product has an effectiveness level of 99.98 to kill S. aureus with a contact time of 30 seconds and 99.99% for a contact time of 60 seconds (Table 2).

Test results (Table 2)

Test microbe: Staphylococcus aureus

Contact Time	**Initial number of microbes in suspension**	**Number of living microbes**	**Presentation of killed microbes**
	CFU/ml	**CFU/ml**	**%**
30 sec	50000000	$1,8 \times 10^3$	99,97
60 sec		$3,0 \times 10^2$	99,99

This study included 35 female respondents with hyperpigmentation post-inflammatory. The mean age of the respondents was 26.34 (4.26) years, the Taylor Hyperpigmentation Scale pre-intervention mean score was 4.89 (0.99) points and the Taylor Hyperpigmentation Scale post-intervention mean score was 3.34 (0.97) points. On the other hand, there were 23 (65.7%) respondents complaining of body odor before the intervention and 11 (31.4%) respondents complaining of body odor before the intervention (Table 3)

Table 3. Characteristics of Research Respondents

Parametric	**N (%)**	**Mean (SD)**	**Med (Min – Max)**
Usia		26,34 (4,26)	27 (19 – 35)
Working • Yes • No	 23 (65,7%) 12 (34,3%)		
Taylor Hyperpigmentation Scale (Pre Intervention)		4,89 (0,99)	5 (3 – 7)
Taylor Hyperpigmentation Scale (Post Intervention)		3,34 (0,97)	3 (2 – 5)
Odors (Pre Intervention) • Yes	 23 (65,7%)		

• No	12 (34,3%)		
Odors (Post Intervention) • Yes • No	 11 (31,4%) 24 (68,6%)		

The results of the non-parametric bivariate statistical test using the Wilcoxon test (Shapiro Wilk < 0.05) showed that there was a significant difference in the Taylor Hyperpigmentation Scale value between before and after the drug formulation (p-value < 0.001). The value of the Taylor Hyperpigmentation Scale before the intervention was 5 (3 – 7) points and the value of the Taylor Hyperpigmentation Scale before the intervention was 3 (2 – 5) points. (Table 4)

Table 4. Taylor Hyperpigmentation Scale values before and after intervention

Parametric	Mean (SD)	Med (Min – Max)	p-value
Taylor Hyperpigmentation Scale (Pre Intervention)	4,89 (0,99)	5 (3 – 7)	< 0,001
Taylor Hyperpigmentation Scale (Post Intervention)	3,34 (0,97)	3 (2 – 5)	

*Statistical analysis using the Wilcoxon test, due to the distribution of data that is not normal according to the Shapiro Wilk test (p-value < 0.05)

The results of the pre-post intervention bivariate statistical test for qualitative data on body odor before and after the intervention, the results showed that there was a significant change in body odor parameters after using the intervention formulation for the better (p-value 0.008) (Table 5)

Table 5. Comparison of armpit odor pre-post intervention

Parametric		Odors Post Intervention				Odds Ratio			*P – value**
		Yes		No		OR	CI 95%		
		N	(%)	N	(%)		Lower	Upper	
Odors Pre Intervention	Yes	8	34,8	15	65,2	1,60	0,335	7,639	0,008
	No	3	25	9	75				

*Statistical analysis using McNemar test (Pre-Post Qualitative Data)

Assessment of side effects with the use of intervention products showed that there were only 1 (2.86%) respondents who experienced complaints of itching and redness (Table 6).

Table 6. Side Effects of Use of Interventions

Parametric	N (%)
Itching	1 (2,86%)

Redness	1 (2,86%)
Skuama	0
Infection	0

4. DISCUSSION

A. HYPERPIGMENTATION ON FEMALE AXILLARY SKIN

Underarm skin darkening is a big consumer issue among the female population of South East Asia (Thailand, Indonesia and the Philippines), and numerous deodorant and antiperspirant products occur on the market, in this region, that promise to whiten the axilla. However, while much is known about the consumer experience and attitude towards underarm darkening, little is known about the characteristics distinguishing pale and hyperpigmented skin in the axillae of individuals in the general SE Asian community. Underarm darkening has been linked to postinflammatory hyperpigmentation (PIHP), on the premise that strongly pigmented skin has a larger rise in colour, following inflammatory damage or stimulation, than non-pigmented or weakly pigmented skin. PIHP is defined by the development of dermal melanophages, following hypermelanogenesis or physical injury in the epidermis, with pigment seeping into the dermis and being consumed by macrophages, subsequent to a major inflammatory event. We suggested that axillary darkening is moderate PIHP, defined by increased epidermal melanin, after stimulation or mild irritation of skin. A range of stimulatory stimuli can be implicated, including axillary hair plucking, the most prevalent type of depilation in the area. Other probable contributing variables include the movement, stretch and contraction of pigmented axillary skin, the rubbing of clothing on skin, and physical stimulation from washing and drying the underarm. [4]

B. Host–pathogen interactions

The majority of study has been done on the interactions between microbes and their hosts, which are responsible for or arise from infectious processes. Microbes and disease have a one-to-one connection in skin, with inflammation being the most immediately apparent sign of infection. Most of what we know about the skin immune system comes from looking at interactions like these, which shows the usefulness of this basic paradigm while also foreshadowing its drawbacks. As we'll see, the majority of microbe–host interactions on the skin are far more modest; for example, traditional pathogens commonly reside asymptomatically on the skin's surface. [13]

Skin infections caused by S. aureus are among the most costly and common health problems in the world. Even though S. aureus colonizes more than 30% of healthy people without symptoms, it can cause a wide range of infections: some are limited to a single hair follicle (furuncle), others involve subcutaneous tissues (cellulitis), and the most serious feature is potentially fatal penetration into any organ in the body, including bone (ostemyelitis), bloodstream (bacterial sepsis), and heart valves (cardiac sepsis) (bacterial endocarditis). Atopic dermatitis and systemic lupus erythematosus, both of which affect the kidneys and the skin, have been related to Staphyloccocus aureus in the past. [14–16]

Anti-phagocytic and antikilling surface chemicals, superantigens, and immune evasion proteins all exist in S. aureus, making it a dangerous pathogen with many virulence characteristics. It is believed that S. aureus isolates form biofilms on the skin of people with atopic dermatitis, where

they produce proteases that degrade the body's own AMPs such cathelicidin LL-37. The host has evolved systems to keep S. aureus out at all levels of the epidermis and subcutaneous tissue. The innate immune response is aided by adipose tissue under the dermis, which, in addition to a diverse array of AMPs, may play a role. Local pre-adipocytes proliferate fast after an epidermal barrier breach and subsequent S. aureus infection, expanding the subcutaneous fat layer and increasing AMP cathelicidin production.[13]

All S. aureus species share certain virulence and immune evasion characteristics, although strains differ greatly. S. aureus USA300, a methicillin-resistant strain that can thrive in the acidic environment of human skin and resist polyamines, for example, has an arginine catabolic mobile element, which may explain why this strain is so common in skin and soft tissue infections. Certain strains of S. aureus have also been linked to more severe atopic dermatitis, according to recent study. These strains are also capable of generating skin inflammation in mice independent of the host's genetic predisposition, the research found. Using genus- or species-level data to characterize microbiome composition, this study revealed that the most popular method fails to identify significant functional differences across strains, which can result from small gene gain or loss events or even variations in gene expression. [15,17]

C. ODORS AND BACTERIAL

Affecting the axillary, vaginal, and foot regions, bromhidrosis is a bothersome condition characterized by bad body odor. Sweat glands of all three types have a role in the emergence of this disease, sweating excessively, then breaking down sweat components due to bacterial action results in an unpleasant perspiration smell. The most common kind of bromhidrosis is apocrine, which develops after puberty rather than eccrine. Many volatile chemicals are produced by the breakdown of the apocrine sweat, including ammonia and short-chain fatty acids, such as the C7 branched and unsaturated acid (E)-3-methyl-2-hexenoic acid (E3M2H). According to legend, it has an overpowering stink. Natsch et al. revealed that commensal Corynebacterum species' N-AGA releases odorous E3M2H and its hydrated counterpart, (R)/(S)-3-hydroxy-3-methylhexanoic acid ((R)/(S)-HMHA), from glutamine conjugates (present in axilla secretions). HMHA has been shown to be the most prevalent kind (which has a rotten, cheesy odor). The (S)-isomer of 3-methyl-3-sulfanylhexan-1-ol ((R)/(S)-MSH), which has an oniony, clary sage-like odor, is another particle that causes axillary malodor. Eccrine bromhidrosis has also been related to a variety of other conditions. Garlic or onion consumption, microorganisms breaking down keratin, metabolic issues and hyperhidrosis are examples of these. [8–12]

D. EFFECTIVENESS OF THE COMPOSITION IN INHIBITING MICROORGANISMS, ELIMINATING BODY ODOR, AND REDUCE HYPERPIGMENTATION

Cosmetics for skin cleaning may be classified into four types: water-based, oil-based, solid, and scrub-based. Astringent lotion, for example, is a water-based cleaner. A surfactant must be added to the water to give it a powerful cleaning ability, but the type of surfactant used must be carefully considered because it might result in dry and irritated skin. Oil-based cleansers, such as cleansing creams and cleansing milks, are excellent in removing oil-soluble pollutants and typically have higher cleaning power. After evaporating the solvent, the solid powder that forms from the solid cleansers absorbs dirt and removes it. People who cannot tolerate soap yet are more likely to

develop allergies can benefit from this treatment. When it comes to removing dead skin cells, only scrub cleansers are effective.[18]

Cleaning formulas rely heavily on surfactants as their primary active ingredient. Perfumes, structures, emollients, and humectants are common additions to cleaners. The price of soap is mostly determined by the cost of the fragrance. The structure helps to keep the soap in its solid state. In order to counteract the drying effects of surfactants, emollients are often utilised. To keep the skin moisturized, humectants are used. Pathogenic bacteria can be eliminated by including disinfectants in the cleaning process..[19]

It was first identified as a quasi-drug in Japan in 1962 and as a cosmetic ingredient in 1967 as the photosensitizer 3-heptyl-4-[3-heptyl-4-methyl-2(3H)-thiazolylidene)methyl]-4-methylthiazolium iodide. Pionin is extensively utilized in anti-acne treatments because of its great antibacterial activity and ability to destroy Proprionibacterium acnes. According to one study, compared to a placebo, using soap containing 0.005 percent pionin decreased the frequency of papules and pustules in acne. In cosmetics, Pionin works well even at low doses.[20]

Aloe Vera is a plant native to the tropics that has several dermatological applications. As an anti-inflammatory, hydrating, and anti-aging agent, topical Aloe Vera helps heal wounds while also protecting them from the harmful effects of the sun and gamma radiation they get. For the most part, aloe vera cream is used topically to treat burns, eczema, and other skin conditions. [21,22]

Glutathione is a tripeptide consisting of cysteine, glycine, and glutamate which functions as an antioxidant. Reduced glutathione (GSH) has a skin whitening effect through tyrosinase inhibition. Based on the study found that topical 2% glutathione in the form of a lotion given twice a day for 10 weeks whitens the skin significantly. In addition, the skin given this preparation becomes softer and not dry. [23–25]

Lavender (Lavandula officinalis) is an herb that is widely used for aromatherapy. Lavender essential oil has anti-inflammatory, analgesic, antibacterial and antifungal effects. Lavender is also thought to be helpful in the management of anxiety and depression. [26–31]

The formulation of the cleanser solution was made by mixing pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06%, then added aquabides as a solvent. up to 100% concentration. The cleaning solution is mixed with tissue sheets measuring 18-54 cm x 16-48 cm which are processed in a machine. The output is body tissue that has been wrapped in a special container. The container states how to use it.

The formulation of this cleanser has an antimicrobial effect so that it can effectively cleanse the skin of disease-causing germs and prevent the formation of unpleasant body odors. This formulation can also whiten the skin of the armpits, where the armpit area is usually dark in color. By making a cleanser in the form of armpit tissue, it provides added value from the practical side. The present invention is intended for various situations where it is difficult to take a shower such as when traveling, caring for the sick or while working overtime. This invention is expected to provide convenience for cleaning the body, preventing body odor and making the body feel fresh again so as to increase productivity.

5. CONCLUSIONS

The axillary tissue cleansing formulation contains pionin 0.08-0.12%, aloe vera 1.6-3%, glutathione 2.4-5%, and lavender 0.04-0.06%, proven to inhibit bacterial growth in the laboratory. On the other hand, clinically,

the use of this intervention was found to be able to reduce or fade the symptoms of post-inflammatory hyperpigmentation and relieve body odor with very minimal side effects.

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