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Research Article

In-vitro antibacterial assessment of a deodorant roll-on formulated from Gatra Dourgandhyanashana Lepa with underarm swab samples

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Abstract

Introduction: *Sweda* (sweat), a bodily exudation linked to fat tissue, helps maintain skin moisture but excessive sweating causes bad odor and itching. In Ayurveda, various formulations are outlined to reduce *deha dourgandhya* (bad odor). One such remedy is the *gatra dourgandhyanashana lepa*, comprising *vasa* (*Adhatoda vasica* Nees.) and *shankha* (conch shell). In this study, deodorant the modified form of *gatra dourgandhyanashana lepa* was tested for antibacterial activity. Materials and methods: The bacterial load before and after application of deodorant was assessed by measuring the total viable count using in-vitro serial dilution of underarm swabs. The obtained results of before application (BA), after application 2hrs (AA2) and after application 4hrs (AA4) were subjected to statistical analysis. Results and Discussion: Descriptive statistics showed mean bacterial counts of 6188.89 ± 4088.534 (BA), 2557.78 ± 2021.273 (AA2), and 2166.67 ± 1161.895 (AA4). The Friedman test ($p = 0.034$) indicated a statistically significant reduction in microbial load in the study samples between at least two of the time points. Wilcoxon's test revealed AA4 showed significant reduction ($p = 0.002$), but AA2 did not ($p = 0.049$), suggesting deodorant's effectiveness at 4 hours. Conclusion: The present study demonstrated a decrease in bacterial counts, with the effect persisting four hours after the application of the deodorant roll-on.

Keywords: *Deodorant, Lepa, Gatradourgandhya, Body odour, Total viable count, Serial dilution*

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Introduction

Sweda (sweat) is one among *trimala* (three types of excreta), it is the excreta of fat tissue (1) and liquid that exudes from hair follicles because of body heat (2) Sweat helps in maintaining moisture of skin but excessive sweating causes bad odor and itching. (3) Body odor, resulting from the bacterial decomposition of sweat, (4) can be disagreeable and socially unacceptable. It can also be aggravated by factors like intense physical activity, dietary choices, emotional states such as anger or grief, as well as changes in temperature. This phenomenon can have adverse effects on both the mental and physical well-being of an individual.

Fragrances, perfumes, deodorants, and antiperspirants are commonly used to mask body odour while fulfilling multiple purposes, including regulating sweat, improving personal hygiene, enhancing confidence in social settings, ensuring comfort, upholding a professional image, and adhering to cultural norms. However, even some of crystal deodorants found in the market contain aluminum salts, which have been linked to skin irritation and allergic responses. (5,6) So, herbal products and other natural alternatives for self-care are preferred in the current era.

In Ayurveda while explaining seasonal regimen (7) of autumn and summer and in the person of *pitta* constitution, (8) usage of garlands of aromatic herbs and applications are mentioned. Various formulations are outlined to reduce *deha dourgandhya* (bad odor). One such remedy is the *Gatra dourgandhyanashana lepa* (9,10,11,12) comprising *vasa swarasa* (leaf juice of *Adhatoda vasica* Nees.) and *shankha churna* (conch shell ash). However, preparing this *lepa* (paste) fresh can be inconvenient. With modern advancements in technology, there is a preference for products that require minimal effort and ease of use. As a result, the *Gatra dourgandhyanashana lepa* has been modified into a deodorant roll-on, and its modified form has been tested for antibacterial activity against odor-causing bacteria.

Objective

To assess anti-bacterial property of deodorant roll-on formulated from *gatra dourgandhyanashana lepa.*

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Materials and methods

Materials

The materials used in the present study include:

Equipment's: Test tubes, Pipette, Petri dish, Conical flask, Swab

Media and reagents: 1N physiological saline, plate count agar,

Methods

Preparation of deodorant roll-on

Authentication of *shankha* was done based on physical appearance like color, shape, diaphaneity, external surface, opening and columella. Also, studied with physical properties mentioned in books of *Rasashastra* (Iatro-chemistry and Ayurvedic pharmaceutics). Authentication of *vasa* was based on morphological characteristics of the leaves of *vasa*. It was also compared with standard herbarium.

Vasa arka (distillate of *Adhatoda vasica* Nees. leaf), *shankha bhasma* (conch shell ash), and emulsifying wax were heated in a double boiler, stirred until the wax melted. Essential oil was added for fragrance, and the mixture was stirred with a magnetic stirrer until it thickened and cooled. It was then packed in airtight glass roll-on bottles.

Inclusion and exclusion

Patients with in the age of 18-40 years, with symptoms of excessive sweating and bad odour, ready to sign written consent were included and people having skin disorders and armpit sensitivity were excluded from the study.

Study methodology

The total viable count of bacteria assessed by serial dilution (13,14) of underarm swab before and after application of deodorant. The study was explained to the volunteers, and those who expressed interest in participating were approached for the same.

The wet swab method was used to collect samples from a marked area on the volunteer's armpit. A sterile swab, moistened with 1 ml of 1N physiological saline, was rotated clockwise and counterclockwise on the area. The swab was then placed back in the swab cover containing saline. One swab was collected before deodorant application (BA), and two more were collected after 2 hours (AA2) and 4 hours (AA4) post-application at maximum possible aseptic condition. All samples were stored in sterile covers and labeled accordingly.

9ml of 1N physiological saline was added into each of 7 test tubes with sterile pipette. Test tubes were labelled indicating dilution factor (10^{-1} to 10^{-7}). 1ml of inoculum was added to the test tube indicating10^{-1} concentration and mixed gently. Further dilution i.e., 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} was done by transferring the inoculum subsequently to each test tubes with the help of sterile pipette. Each swab content was inoculated into test tube and subjected to serial dilution in the same method.

Plate Count Agar was used as the media. Fresh media was prepared each time using the standard method, with sterile, autoclaved distilled water. Two sterile petri dishes (Duplicates) labelled with control number were taken and 1 ml of initial dilution (10^{-1}) was added with sterile pipette. 15ml of plate count agar at 44℃ to 47℃ was added into each plate and rotated in all direction. Petri plates were closed to ensure vacuum shunting to minimize cross-contamination from external sources. Mixture was allowed to solidify in cool horizontal surface and plates were incubated in invert position. Same procedure was followed for other dilutions i.e., 10^{-2} to 10^{-7}. After proper incubation at 30℃ for 24 to 36hrs, plates were taken out. Petri plates were subjected to photo documentation and the plates having fewer than 300 colonies were retained. Distinct colony forming units were counted under colony counting equipment and following formula was used for calculation. Every 1,000 microbes counted would be 1 X $Log^{10}3$ (expressed in terms of power. i.e.1 X 10^3)**.**

$$N = \frac{\Sigma C}{V \times [n_1 + (0.1 \times n_2)] \times d}$$

Where: N= Total colony forming units; C= Number of colonies; V= Volume of inoculum; n_1 and n_2 = Number of plates considered; d = Dilution factor.

Assessment Criteria

Intervention design

Criteria	Trial drug group (after 2hrs and 4hrs)
Sample size	9
Dose	0.5ml
Number of administrations	Single
Assessment	2hrs and 4hrs
Number of follow-ups	None

Statistical analysis

The data were analyzed using mean ± SD (standard deviation). To assess the normality of distribution Shapiro-wilk test (15) and Q-Q plots were used. To assess the significance of differences between the means of colony-forming units, the Friedman test (16) was used. For determining the precise differences, the Wilcoxon signed-rank test (17) with Bonferroni correction (18) was applied to various group combinations.

Observations and Results

Table 1: Colony count in serial dilution

Volunteer no.	Dilution	Before Application (BA)		After Application 2hrs (AA2)		After Application 4hrs (AA4)	
1	10^{-1}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	10^{-2}	119	121	33	30	31	34
	10^{-3}	6	9	6	4	3	5
2	10^{-1}	TNTC	TNTC	10	13	241	226
	10^{-2}	53	49	1	3	24	25
	10^{-3}	6	7	0	0	1	3
3	10^{-1}	TNTC	TNTC	165	178	TNTC	TNTC
	10^{-2}	81	60	19	22	31	34
	10^{-3}	15	8	0	0	3	0
4	10^{-1}	TNTC	TNTC	95	97	235	221
	10^{-2}	107	101	8	5	44	35
	10^{-3}	52	48	0	0	9	8
5	10^{-1}	TNTC	TNTC	96	120	TNTC	TNTC
	10^{-2}	41	44	11	17	52	30
	10^{-3}	4	5	0	0	7	3
6	10^{-1}	295	292	288	280	53	47
	10^{-2}	67	88	112	105	4	9
	10^{-3}	12	15	24	29	0	0
7	10^{-1}	293	295	93	93	115	123
	10^{-2}	63	52	32	39	21	14
	10^{-3}	6	11	0	0	0	0

8	10^{-1}	236	231	TNTC	TNTC	101	89
	10^{-2}	41	117	53	68	21	34
	10^{-3}	15	23	8	11	0	0
9	10^{-1}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	10^{-2}	35	40	53	48	11	18
	10^{-3}	2	5	4	7	0	0

*TNTC-Too numerous to count

Table 2: Colony forming unit/swab

Volunteer No.	BA	AA2	AA4
1.	1.2×10^4	3.3×10^3	3.3×10^3
2.	5.2×10^3	1.2×10^2	2.4×10^3
3.	7×10^3	1.8×10^3	3×10^3
4.	1.4×10^4	9×10^2	2.5×10^3
5.	4.2×10^3	1.1×10^3	4×10^3
6.	3.5×10^3	3.6×10^3	5×10^2
7.	3.2×10^3	1.2×10^3	1.2×10^3
8.	2.8×10^3	6×10^3	1.1×10^3
9.	3.8×10^3	5×10^3	1.5×10^3

Statistical analysis

Table 3: Descriptive Statistics and test for normality

Group	N	Mean	Std. Deviation	Shapiro-Wilk test
				Sig.
BA	9	6188.89	4088.534	0.015
AA2	9	2557.78	2021.273	0.399
AA4	9	2166.67	1161.895	0.833

Table 4: Friedman test

	Mean Rank		Test statistics (N-9)
Before	2.67	Df	2
A4	1.56	Chi-Square	6.588
A2	1.78	Exact Sig.	0.034

Table 5: Wilcoxon signed rank test

		N	Mean Rank	Test statistics	
				Z	Exact Sig. (1-tailed)
AA2-BA	Negative Ranks	6[a]	6.17	-1.718[g]	0.049
	Positive Ranks	3[b]	2.67		
	Ties	0[c]			
	Total	9			
AA4-BA	Negative Ranks	9[d]	5.00	-2.666[g]	0.002
	Positive Ranks	0[e]	0.00		
	Ties	0[f]			
	Total	9			

a. AA2 < BA, b. AA2 > BA, c. AA2= BA, d. AA4< BA, e. AA4 > BA, f. AA4 = BA. g=Based on positive ranks.

Discussion

The *gatra dourgandhyanashana lepa* is a blend of herbal and mineral ingredients, including *shankha bhasma* (conch shell ash) and *vasa swarasa* (juice of *Adhatoda vasica* Nees. leaf). Since *swarasa* (juice) has a short shelf life and needs to be freshly prepared, it has been replaced with *arka* (distillate) in deodorant roll-on, which has a longer shelf life (19), is sterile, and does not stain clothes.

The swabs were collected before and after the application of deodorant. The collection of swabs includes marking of a designated area on the armpit to standardize the sample collection process. This targeted region and collection in two directions ensures that the sample accurately reflects the underarm skin microflora and maintains consistency across collections. The collected swab was subjected to serial dilution which helps in reducing a dense population of bacteria to a more manageable level, and the even distribution helps in visible quantification of bacteria.

The obtained results were subjected to normality test. The Shapiro-Wilk test results indicate that the data for the BA is significantly non-normally distributed, suggesting deviations from normality. Conversely, the AA2 and AA4 groups did not show significant deviations from normality.

As the sample size is less, BA group didn't show normal distribution, non-parametric test with exact statistics was applied. Exact statistics helps accuracy with small sample and reliability of results (20).

In descriptive statistics, the mean ± S.D of BA, AA2 and AA4 are 6188.89±4088.534, 2557.78±2021.273 and 2166.67±1161.895 respectively. The mean bacterial count decreases from BA to AA2, and then further decreases AA4. This suggests reduction in variability over time. In Friedman test, the Exact Significance value is 0.034, this indicates a statistically significant reduction in microbial load in the study samples between at least two of the time points.

Since the objective was to know the reduction in bacteria after the application of deodorant when compared to that of before, in Wilcoxon-signed rank test exact significance (1-tailed) was considered. With a Bonferroni adjustment, the significance level is 0.025. The exact significance (1-tailed) for the AA2 versus BA comparison is 0.049 which is greater than 0.025, meaning it is not statistically significant. However, the exact significance (1-tailed) for the AA4 versus BA is 0.002 is less than 0.025, indicating statistically significant result. Therefore, the deodorant is effective in reducing bacterial counts after 4 hours, but not after 2 hours, when adjusted for multiple comparisons.

The *Gatra dourgandhyanashana* deodorant is a formulation combining *vasa* (*Adhatoda vasica Nees.*) (21) and *shankha* (conch shell) (22), which may work to mitigate the *ushna veerya* (heat) and *snehamsha* (oily nature) of pitta by providing *sheeta* (cooling) and *ruksha* (drying) properties. *Vasa*, with its *tikta* (bitter) and *kashaya* (astringent) tastes, may assist in reducing the *dravata* (liquid nature) of pitta, while the *sheeta grahi* (cooling and retaining) nature of *shankha* can help in reducing the *saratva* (mobility) of pitta. Moreover, the *ksharata* (alkaline) of *shankha* may aid in alleviating the *amlata* (sourness) of pitta while addressing the *snigdhata* (unctuousness) of fat tissue. By counteracting the *snigdha* (unctuous) and guru (heavy) qualities of *meda dhatu* (fat tissue) with *laghu* (light) and *ruksha* (dry) properties, the deodorant helps reduce the *udakamsha* (liquid) of *meda* (fat) and decreases the influence of pitta. Consequently, this formulation may contribute to restoring balance in *swedotpatti* (sweat production) and reducing *dourgandhya* (bad odor).

Conclusion

The present study indicated a reduction in bacterial counts, with the effect remaining consistent four hours after application of deodorant roll-on. *Gatra dourgandhyanashana* deodorant formulation, combining *vasa* and *shankha*, showed antibacterial activity.

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Conflict of interest: None

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