Original Article

Deodorant effects of a sage extract stick: Antibacterial activity and sensory evaluation of axillary deodorancy

Mohammad Ali Shahtalebi[1], Mustafa Ghanadian[1,3], Ali Farzan[2], Niloufar Shiri[3], Dariush Shokri[4], Syed Ali Fatemi[5]
Department of Pharmacognosy, [1]Department of Pharmaceutics, Isfahan Faculty of Pharmacy, [2]Department of Biostatistics and Epidemiology, School of Health, [3]Isfahan Pharmaceutical Sciences Research center, [4]Nosocomial Infection Research Center, [5]Department of Pharmacology, Isfahan Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Deodorant products prevent the growth and activity of the degrading apocrine gland bacteria living in the armpit. Common antibacterial agents in the market like triclosan and aluminum salts, in spite of their suitable antibacterial effects, increase the risk of Alzheimer's disease, breast and prostate cancers or induce contact dermatitis. Therefore, plant extracts possessing antibacterial effects are of interest. The aim of the present study was to verify the *in vitro* antimicrobial effects of different sage extracts against two major bacteria responsible for axillary odor, and to evaluate the deodorant effect of a silicon-based stick containing sage extracts in different densities in humans. **Materials and Methods:** Different fractions of methanolic extract of *Salvia officinalis* (sage) were evaluated on a culture of armpit skin surface of volunteers through agar microdilution antimicrobial assay. Then, randomized, double-blind placebo-controlled clinical trial with the best antibacterial fraction was conducted on 45 female healthy volunteers. Participants were treated with a single dose in four groups, each containing 15 individuals: Group 1 (200 μg/mL), 2 (400 μg/mL), 3 (600 μg/mL) of dichloromethane sage extract, and placebo (without extract). A standard sensory evaluation method for the evaluation of deodorant efficacy was used before, and two hours, four hours, and eight hours after single application of a deodorant or placebo (ASTM method E 1207-87 Standard Practice for the Sensory Evaluation of Axillary Deodorancy). **Results:** The data were analyzed with two factors relating to densities and time. In 45 participants with a mean [± standard deviation (SD)] age of 61.5±11.8 years, statistically significant within-group differences were observed before and two, four, and eight hours after deodorant treatment for groups 1, 2, and 3. Groups 1, 2, and 3 had a significantly smaller odor score than placebo after two, four, and eight hours ($P < 0.001$). In a comparison of different deodorant densities, the interaction effect was not significant between deodorant 200 and 400 μg/mL, but was significant between 200 and 600 and between 400 and 600 μg/mL sage extract sticks ($P < 0.001$). Before running the sensory evaluation of the deodorant sticks on the subjects, a rabbit skin patch test was used to demonstrate that the formulation had no irritants. **Conclusion:** A single treatment with a stick deodorant containing dichloromethane sage extract of 200, 400, or 600 μg/mL concentrations was effective in reducing the axillary malodor level compared with the control, in healthy subjects.

Key words: Antibacterial activity, axillary deodorant, sage extract, stick

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INTRODUCTION

Sweat glands secretion is by itself odorless, and armpit malodor is caused by the microbial biotransformation of the odorless secretion into volatile odorous molecules. [1] Therefore, a satisfactory deodorant product could prevent the growth and activity of the degrading apocrine gland secretion bacteria like *Staphylococcus epidermidis* and *Corynebacterium* species.[2] Nowadays, in most deodorant products, antibacterial agents such as quaternary ammonium compounds like triclosan, aluminum salts, and aromatic odor-masking agents are used.[2] Aluminum salts, in spite of their suitable antibacterial effect, increase the risk of Alzheimer's disease and breast and prostate cancers.[3-6] Many of other antibacterial agents found to be effective against skin organisms are irritating or sensitizing.[6] There is also the risk of resistance to ordinary antibiotics. Therefore, herbal extracts possessing antibacterial effects against staphylococci and aerobic coryneforms are alternatively available for the treatment of armpit odor [7-9] Among plants, sage is a good candidate due to the presence of ursolic acid and carnosic acid with suitable antibacterial effects against the *Corynebacterium* species responsible for the sweat odor.[10] It is further known from US Patent number 6139825 that supercritical carbon dioxide (CO_2) extracts of sage as an active ingredient between 0.5 and 5% is used for producing gel or roll-on deodorants. In this study, the impact of sage extract on sweat-decomposing bacteria was evaluated through agar well

Address for correspondence: Dr. Mustafa Ghanadian, Department of Pharmacognosy, Isfahan Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: ghanadian@pharm.mui.ac.ir
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diffusion method. It showed that 1% CO_2 sage extract has a significant inhibiting effect on *Corynebacteriu*m strains and *S. epidermidis*.[11] To the best of our knowledge, this is the first report of sensory evaluation of axillary deodorancy of dichloromethane sage extract in a silicone-based stick formulation in humans to verify the *in vitro* antimicrobial effects of different sage extracts against two major bacteria responsible for axillary odor.

MATERIALS AND METHODS

Plant materials

Aerial parts of *Salvia officinalis* (sage) were collected in July 2012 from the Isfahan province (Iran). The plant material was identified by the Pharmacognosy Department,department, Pharmacy Faculty, Isfahan University of Medical Sciences, Iran, and a voucher specimen was deposited. Shade-dried plant material (200 g) was macerated with aqueous ethanol (4:6) at room temperature for five days. Filtration and under vacuum concentration of total hydroalcoholic extract resulted in a green gum which was partitioned between aqueous methanol and hexane. The defatted methanolic extract was concentrated, dissolved in water, and extracted sequentially with dichloromethane and *n*-butanol. The obtained fractions were vacuum-concentrated and kept in a refrigerator at –20°C.

HPTLC standardization of the sage hydroalcoholic extract

Rosmarinic acid is one of the major components of *S. officinalis* responsible for the observed biological activities.[12] An accurate and repeatable high-performance thin-layer chromatography (HPTLC) method with the help of a TLC scanner was done on the sage extract for the quantification of rosmarinic acid.[13] Briefly, 100 mg of the concentrated hydroalcoholic extract of the *S. officinalis* was mixed thoroughly with 1 mL methanol: Water (70:30) repeatedly three times. The combined extract containing rosmarinic acid material was filtered to 3 mL. The sample was spotted in the form of 1 µL spot width on a prewashed silica gel TLC aluminium foil 60 (20×10 cm with 0.2 mm thickness; E. Merck, Darmstadt, Germany) using a Camag nanomat (CAMAG, Muttenz, Switzerland). A constant application rate of 150 nL/s was employed, slit dimension was kept at 4×0.1 mm, and 20 mm/s scanning speed was employed. The mobile phase consisted of toluene-ethyl acetate-formic acid (5-4-1). Determination was done at 329 nm using a TLC Scanner 3 (CAMAG, Muttenz, Switzerland). A standard calibration curve in the range of 50 to 400 µg/mL for quantitative analysis was prepared using different concentrations of rosmarinic acid (Sigma Aldrich, USA) as standard material (50, 100, 200, and 400 µg/mL). The relationship between the concentration and peak height was measured using the minimum square method (R^2 value). Validation of the HPTLC method was calculated as the percent recovery of spiked extract sample with standard rosmarinic acid at 100 µg/mL concentration. Limit of detection (LOD) and limit of quantification (LOQ) were determined by using the formula based on the signal-to-noise ratio. LOD and LOQ were calculated by using equations, LOD=3 × S/N′ and LOQ=10 × S/N′, where S = signal height, and N′= noise height.[13]

Bacteria preparation

The sage extract was tested *in vitro* on the cultures of *S. epidermidis* PTCC 1114 (Industrial Bacteria and Fungi Collection, Iran) and *Corynebacterium* strain isolated from the armpit skin surface of a volunteer to confirm that the extract was able to reduce the population of axillary bacteria.[14]

Agar microdilution antimicrobial assay

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *Corynebacterium* strains and *S. epidermidis* were determined using agar microdilution assay.[14] The culture of both bacteria was diluted with sterile tryptic soy broth (TSB) to match a McFarland 0.5 turbidity standard, and then further diluted to give a concentration of approximately 1.5×10^6 cfu/mL. Then, 500 µL of bacterial suspensions were added to 10 tubes containing 9 mL of Mueller-Hinton broth media; 500 µL of sage extract with density of 12.8 mg/mL was added to the first tube and serial dilution process was done producing the concentrations of 0.025, 0.05, 0.1, 0.2,0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 mg/mL. A ciprofloxacin disc (4 mg/mL) was used as a standard drug for comparing the antibiotic activity and a medium with micro-organisms was used as positive control.[14]

Sensory evaluation of human axillary deodorancy

A standard method for substantiating deodorant efficacy of personal care products using trained odor judges for the assessment of axillary malodor and indirect sniff method instead of direct sniffing were used.[15-17]

SUBJECTS

This randomized, double-blind placebo-controlled clinical trial was conducted among 45 healthy female volunteers between 20 and 68 years of age. The subjects were screened for axillary irritation prior to acceptance in the study by an expert in cosmetic pharmacology. The study was approved by the Ethics Committee of the Isfahan University of Medical Sciences. Healthy subjects who were able to generate a moderate axillary odor (odor intensity score ≥4.0 and ≤8 with right-left odor difference ≤1.0) with no medical history of allergy to deodorants and/or antiperspirants, no axillary irritation, no disease, and no medication use prior to and following the intervention were included in the study. None of the subjects showed signs of axillary irritation during the test period, and all of the enrolled subjects completed

the study. The participants were treated with a single dose of three sticks with different dosages of 600, 400, and 200 μg/mL dichloromethane sage extract in a silicone-based stick containing propylene glycol and cyclopentasiloxane or placebo (silicone-based stick containing propylene glycol and cyclopentasiloxane without sage extract; Pharmacy School, Isfahan University of Medical Sciences, Iran). Forty-five subjects were randomized using permuted block randomization to one of the three groups, each containing 15 individuals, for deodorant or placebo treatment on the right or left axilla. The deodorant or placebo application was done on the right or left hand but assignment to the right or left remained the same during the study. The subjects and judges were not aware of the treatment assignment so as to blind the study. Written informed consent was obtained from each subject. During the study, the subjects agreed to avoid the use of antiperspirant products for a period of three weeks and deodorant products for two weeks prior to the start of the study and continue this for the entire test duration. They shaved their underarms 24 hours prior to the start of the test and abstained from any underarm shaving during the entire test period. They used only one type of soap without flavor for 14 days before the test and abstained from chewing gum, or using sprays or any odorous materials which might have interfered with the assessment.

Collection and handling of samples

Cotton sterile pads were placed in the volunteers' armpits, displaced after two minutes, and then put away in small capped boxes with the subject's name and right or left armpit marked on them. The odor evaluations were done by three judges on a 10-point scoring model, based on a range of armpit odor from none (0) through moderate malodor (5) to strong malodor (10).[16-18] After the initial evaluation, no (0), 5-10 (none, or strong odor), and those with a significant difference between right and left armpit were excluded. Before the trial, the volunteers were asked to wash their underarms with an odorless soaked pad in 2% simple aqueous soap solution for 10 seconds, clean it with a water-dipped pad, and then dry with a clean towel. Finally, they were instructed to use the deodorant sticks and placebo on their armpits. Randomly, half of the subjects used the sticks on the right side and others on the left, although none could identify the deodorant or the placebo. After two minutes, odorless cotton sterile pads were placed in the underarms and were held by antiallergic tapes. After intervals of two, four, and eight hours of a single application of the deodorant or placebo, the pads were replaced.[16-18]

Rabbit patch test

Before running the sensory evaluation of the sticks on the subjects, a rabbit skin patch test as a primary dermal irritation study was done to ensure that the formulation did not cause any irritation. Albino rabbit species with 2.5 kg weight and aged 1.5 years were selected. The rabbits were shaved with a modernized machine and then depilatory powder placed on the skin. Fifteen minutes later, the skin was washed and dried; 100 μL of the stick sample was applied on the shaved part of the rabbit skin. One, 24, 48, and 72 hours later, the size of red irritated areas were scored according to the following scale: 0-1.5 mm: No irritation; 1.5-2.3 mm: Mild erythema; >2.3: Strong erythema.[19]

Statistical analysis

The results are presented as mean ± standard error. One-way analysis of variance (ANOVA) followed by Dunnett's posthoc comparison was used for multiple between-group comparisons. Within-group comparisons were done using paired sample *t*-test. The data analyzed by repeated-measure design test with two factors relating to method and time. In another repeated-measure design with one factor as the function of time, we also compared the mean of deodorant scores after treatment with the control scores (before treatment). Analyses were performed with the statistical package SPSS version 18 (SPSS Inc., Chicago, IL).

RESULTS

HPLC standardization of the sage hydroalcoholic extract

The retention factor (Rf value) for rosmarinic acid was found to be 0.43 ± 0.018. With the help of the Camag TLC scanner and winCATS software, the calibration curve was determined by linear regression in the range of 50-400 μg/mL. The regression equation was $y = 0.4738x - 3.8635$, where X is the concentration of rosmarinic acid in sample (μg/mL) with the correlation cofactor $R^2 = 0.9993$. The percent recovery was 95%, indicating accuracy of the method. The sage extract was standardized to contain 0.52% ± 0.01 (g/100 g) rosmarinic acids. LOD and LOQ were 15 and 50 μg/mL determined by using the formula based on the signal-to-noise ratio [Figure 1].

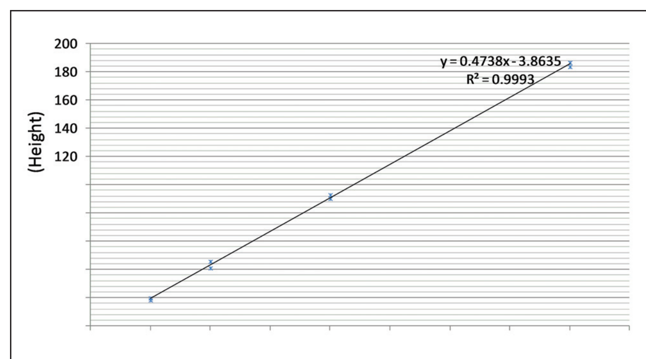

Figure 1: Calibration curve of rosmarinic acid using HPTLC method; using Camag TLC scanner and winCATS software, the calibration curve was determined by linear regression in the range of 50-400 μg/mL; the regression equation was 0.4738x – 3.8635, where X is the concentration of rosmarinic acid in sample (μg/mL) with the correlation cofactor (R^2) of 0.9993.

Agar microdilution antimicrobial assay

Results of MIC and MBC for sage extracts against *S. epidermidis* and *Corynebacterium* using microdilution assay are showed in Table 1. On comparison of total extract and different fractions, MIC values of dichloromethane fraction on *S.epidermidis* and *Corynebacterium* strain with 100 and 200 μg/mL, respectively, were lesser than other fractions. Therefore, dichloromethane fraction with MIC of 200 μg/mL was selected as the antibacterial agent in the deodorant stick formulation.

The reported size of redness on rabbit skin 1, 24, 48, and 72 hours after the patch test was in the range of 0-1.5 mm, indicating no irritation of the prepared stick [Figure 2].

Sensory evaluation of human axillary deodorancy

General mean ± standard deviation (SD) for all study samples for age (years) and weight (kg) was (41.0 ± 11.6) and (61.5 ± 11.8), respectively. There were no statistically significant differences between the groups in terms of basic characteristics.

Placebo and deodorant armpit odor scores after sensory evaluation of deodorant sticks with 200,400, and 600 μg/mL sage extracts are demonstrated in Table 2.

The data were analyzed with two factors relating to densities and time. In within-group analysis, pre and post scores two, four, and eight hours after deodorant treatment for groups 1, 2, and 3 were statistically significant at $P < 0.001$. It means that the deodorant in all three sage concentrations, namely, 200, 400, and 600 mg/mL helped to reduce the odor level in comparison with the control ($P = 0.000$) [Figures 3a-c].

In between-group analysis, there was a significant difference between the mean of placebo and deodorant scores after two, four, and eight hours of using the deodorant ($P > 0.001$), which means that the deodorant with various densities was significantly more effective in reducing the odor level than placebo.

Table 1: MIC and MBC values (μg/mL) of sage total extract and fractions against *Staphylococcus epidermidis* and isolated *Corynebacterium* strain from volunteers' armpits

	Corynebacterium strains		*Staphylococcus epidermidis*	
	MBC	MIC	MBC	MIC
Total extract	12,800	3200	6400	1600
Dichloromethane fraction	800	200	>1600	100
Aqueous fraction	>12,800	6400	>3200	400
Butanol fraction	6400	3200	>12,800	1600

MBC= Minimum bactericidal concentration; MIC= Minimum inhibitory concentration

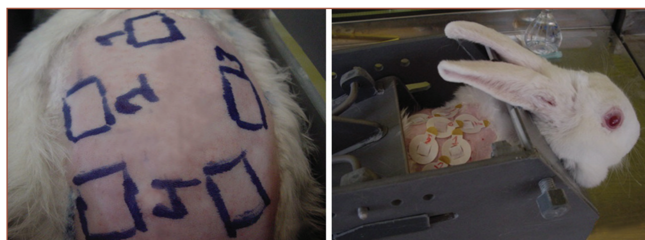

Figure 2: Areas of application of patch sites on backs of rabbits: 1) sage extract sticks, 2) untreated gauze patch as negative control, 3) 1% sodium lauryl sulfate as positive control, 4) placebo or vehicle control

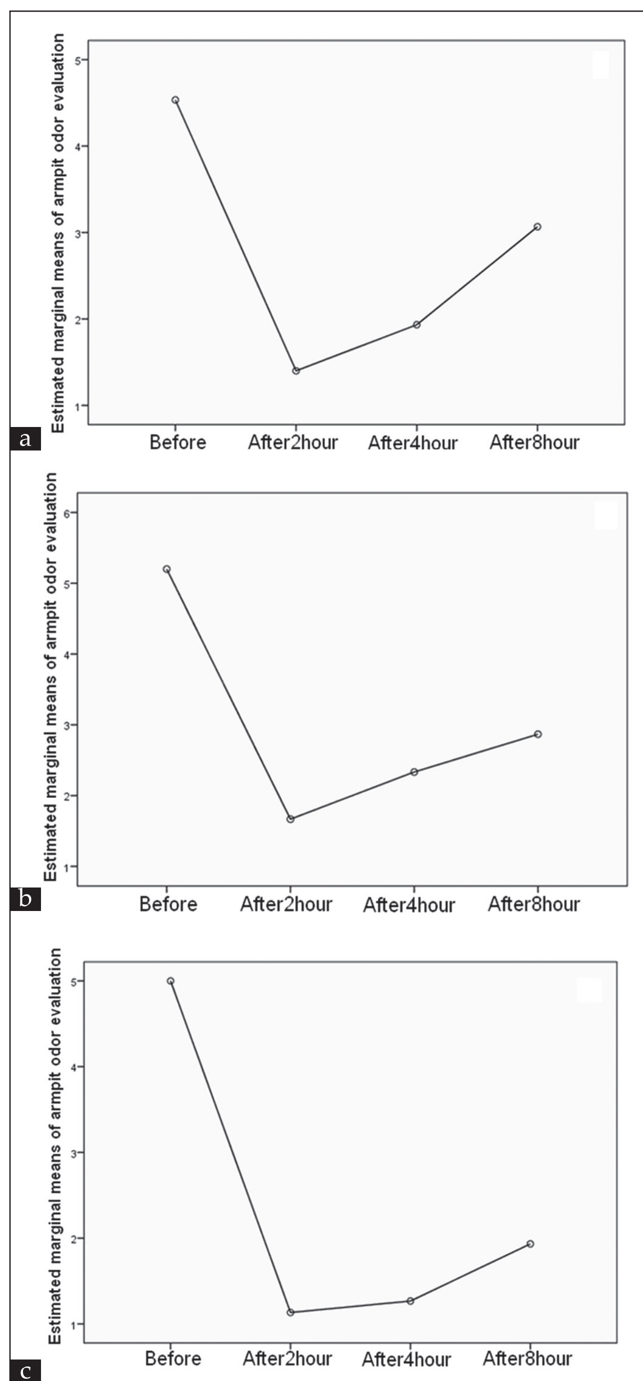

Figure 3: Sensory armpit odor evaluation of placebo or deodorant sticks with (a) 200 μg/mL sage extract, (b) 400 μg/mL sage extract, and (c) 600 μg/mL sage extract

Table 2: Placebo and deodorant armpit odor scores after sensory evaluation of deodorant sticks with 200, 400, and 600 μg/mL sage extracts

Group	Time	Deodorant (mean±SD)	Placebo (mean±SD)	Paired differences	*P* valuea
Deodorant 200	Before	4.53±0.74	4.51± 0.71		
	After 2 hours (t=14.55, *P* <0.001)**	1.40±0.63	2.60±0.99	1.2±0.68	0.000**
	After 4 hours (t=10.22, *P* <0.001)**	1.03±0.80	3.33±1.05	1.4±0.83	0.000**
	After 8 hours (t=4.56, *P* <0.001) **	3.07±1.03	4.27±0.59	1.2±0.86	0.001*
Deodorant 400	Before	5.20±1.01	5.20±1.01		
	After 2 hours (t=13.82, *P* <0.001) **	1.67±0.98	4.27±1.22	2.73±0.88	0.000**
	After 4 hours (t=13.32, *P* <0.001) **	2.33±1.05	4.93±1.03	0.38±0.78	0.000**
	After 8 hours (t=8.64, *P* <0.001) **	2.87±1.19	5.30±1.12	4.13±0.83	0.000**
Deodorant 600	Before	5.00±1.07	5.00±1.07		
	After 2 hours (t=14.13, *P* <0.001) **	1.13±0.35	3.87±0.84	2.73±0.88	0.000**
	After 4 hours (t=15.04, *P* <0.001) **	1.27±0.46	5.07±0.70	3.80±0.78	0.000**
	After 8 hours (t=13.44, *P* <0.001) **	1.93±0.46	6.07±1.03	4.13±0.83	0.000**

[a]Between-group comparisons significant at: **P* <0.01, ***P* <0.001 versus placebo
[b]Within-group comparisons significant at: **P* <0.01, ***P*<0.001 versus before treatment

ANOVA results showed that there were statistically significant between-group differences after two hours (F = 9.99; *P* < 0.001), after four hours (F = 4.77; *P* < 0.001), and after eight hours (F = 18.17; *P* < 0.001) versus placebo. Groups 1, 2, and 3 had significantly lower odor scores than placebo after two, four, and eight hours (*P* < 0.001).

In a comparison of different deodorant densities, the interaction effect was not significant between 200 and 400 μg/mL. It means the change between the mean deodorant scores were the same at various times of observation, but the interaction effect between 200 and 600 (t = 4.75, *P* = 0.000) and between 400 and 600 (t = 5.22, *P* = 0.000) were significant. It means that the change between the mean of deodorant scores of 200 and 400 versus 600 were different in relation to time.

Comparison between pre and postplacebo treatment has shown significant deodorancy effect after two hours (*P* = 0.01), but the deodorancy effect was not significant after four and eight hours of treatment. So, the observed sniff test results should be taken as a combination of the antibacterial effects exerted by the stick constituents and the sage extract. It is also important to note that the product contained not only sage extract as an active ingredient, but that some ingredients of the stick formulation also had antibacterial properties. Propylene glycol, which acts as carrier in the formulation, can contribute to the deodorancy effects.[17]

DISCUSSION

The results of the MIC for sage extract against *S. epidermidis* and *Corynebacterium* species based on microdilution assay showed that dichloromethane fraction with MIC of 200 μg/mL could be used as an antibacterial agent against two major bacteria responsible for underarm malodors. These results were confirmed through another report of the *in vitro* growth-inhibiting properties of sage extract against underarm bacteria, such as *S. epidermidis* and *Corynebacterium xerosis* which have been reported to reduce armpit odors.[20]

The results of the sensory evaluation panel showed significant reduction in malodor scores from 4.53 ± 0.74 to 3.07 ± 1.03, 5.20 ± 1.01 to 2.87 ± 1.19, and 5.00 ± 1.07 to 1.93 ± 0.46 after eight hours of deodorant 200, 400, and 600 treatments, respectively. In comparison with the literature, the results observed with deodorant 600 were more consistent with regular deodorants in the market.[21] The results were also comparable with a similar study with hops extract in trials on humans, in which malodor scores dropped from 6.28 to 1.80 after eight hours of deodorant application.[17]

On comparing the safety of market deodorants and sage extract, aluminum-containing deodorants were reported to induce contact dermatitis due to denaturing epidermal keratin.[22] Hydroxyisohexyl-3-cyclohexene carboxaldehyde (HICC) known as Lyral used in more than 50% of the marketed deodorants is also a frequent allergen, but the sage stick deodorant in all densities was well tolerated without any irritation report.[23] In one study on 14 patients using HICC-containing deodorants, all of them developed unilateral eczema, whereas controls were all negative.[24] Moreover, permeability of armpit membranes to deodorants containing aluminum ions causes reactive oxygen species (ROS). Increased levels of ROS promote cerebral accumulation of extracellular amyloid ß-plaques. Amyloid ß in the brain plays an important role in the development of Alzheimer's disease and mediated neurodegeneration.[5,25] A preliminary study on the dermal

absorption of aluminium from deodorants showed that a small quantity of the applied aluminium was absorbed through the armpit skin.[24] Even though this amount is not significant in a single application, there is an increasing risk of Alzheimer's disease or breast cancer following the absorption of aluminium ions after the extended use of aluminium-containing deodorants.[24-26] There are also reports of ventricular fibrillation or fatal reports following inhalation of deodorant sprays.[27,28] So, replacement by herbal extracts with acceptable antibacterial effects like sage extract could reduce the risk of side effects or toxicities due to the extended use of marketed deodorants.

In summary, there are few clinical trials on natural deodorants to support their efficacy; so, they are probably considered as inefficacious. This is also the first report with sage extract used as deodorant in a stick formulation. Therefore, evaluation of the *in vitro* antibacterial activity of the sage extract and the evaluation of its odor-reducing capacity by a sensory evaluation panel on human subjects was employed to verify its deodorant performance.

The limitation of this study was the sample size. Although the data had normal distribution, a larger number of participants will increase the statistical precision and reduce the standard errors.

Authors' contributions

NS carried out the design and co-ordinated the study and participated in most of the experiments and in manuscript preparation. MS planned, supervised, and conducted the experimental procedures. AF assisted in data and statistical analysis and participated in manuscript preparation. MG contributed in data analysis and writing and finalizing the manuscript. DS provided help for antibacterial experiments. SF, an expert in cosmetic pharmacology, helped with experimental procedures. The authors have read and approved the content of the manuscript.

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