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In vitro assessment of some deodorant ingredients and determination of malodor inhibiting potentials of ascorbic acid, orlistat and mastic gum

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ABSTRACT

Axillary microbial flora has a role in sweat odor formation by transformation of odorless natural secretions into volatile odorous molecules. From 190 axillary isolates obtained from healthy volunteers, 97 isolates, representing three genera; *Stahylococcus* (39), *Micrococcus* (13), *Corynebacterium* (45) were screened for their ability to produce malodorous metabolites from testosterone oenanthate ester andhydroxyprogesterone caproate by sensory, and chromatographic assessments. Only lipophilic *Corynebacterium*spp(*C. jeikeium* and *C. macginleyi*) and *Staphylococcus capitis*isolates were able to metabolize testosterone and hydroxy progesterone esters, with malodorous metabolites from the former only.Quantification of the yield of conversion of substrates into metabolites by the action of *C. jeikeium* was carried out using gas chromatography/mass spectrometryGC/MS. The odourous metabolites are suspected to be Androstadienone and 5-α-dihydrotestosterone, based on mass spectra and previously reported data. The effect of some selected material as potential deodorants on such metabolic activities was studied. In the absence of inhibitor, 99.6% of testosterone ester substrate was converted into metabolites. Ascorbic acid and orlistat dramatically inhibited testosterone ester metabolism allowing only 6.9 %, and 0.6 %conversion, respectively. While mastic gum allowed 84.3% conversion, no malodor was observed. These three components have the potentials to be used as deodorants.

INTRODUCTION

Overproduction of sweat, sweaty skin and body odors are unpleasant for many social groups. Deodorants are designed to combat malodor generated from bacterially modified sweat. Sweat is normally a clear odorless liquid containing odorless C3-fatty acid [(iso) butyric acid, isovaleric acid and propionic acid] triglycerides, which liberate odorous free fatty acid upon hydrolysis by bacterial esterases (Holzle, 2002; Pierard *et al.,* 2003). Androgenic steroids such as 16 androstenes, 5α-androstenol and 5α-androstenone secreted as odorless soluble salts are liberated by hydrolytic ß-lyaseexoenzymes of bacteria as odorous volatile steroids (Gower *et al.,* 1997 a; Starkenmann *et al.,* 2013; Zeng *et al.,* 1991; Zeng *et al.,* 1992). Although the antimicrobials commonly employed as axillary deodorants have excellent safety profiles (Bhargava and Leonard, 1996), it has been argued that disruption or modification of this normal skin flora may predispose to opportunistic infections (Leyden *et al.*, 1987). Furthermore, concerns aboutthe spread of resistant subpopulations of bacteria and about the release of antimicrobials into the environment have effectively precluded the widespread use of these agents in some European countries. For these reasons there have been very real demands for effective non-antimicrobial routes to underarm deodorancy. A number of approaches have been attempted to interfere with the bio-transformational steps leading to the production of odorous compounds.

The present study aims to screen for and evaluate the malodor inhibiting ability of some ingredients of deodorant formulation and some chemicals or natural products for their potential deodorant activities.

MATERIALS and METHODS

Specimens collection

Axillary swabs obtained by rubbing sterile cotton swab against the axilla were collected from adult healthy volunteers, who were receiving no medication or using any form of deodorants or antiperspirants prior to specimen collection with at least forty eight hours (American Society for Testing and Materials; ASTM 1988). The swabs were immediately cultured on selective and nonselective media (Haustien*et al.,* 1993) and the isolates were identified according to Koneman *et al.* (1997a).

Screening for malodor producing isolates

Fifteen milliliters Trypticase soy broth medium (Oxoid, Basingstoke, Hampshire-England) containing 0.1% Tween 80 and 5 mg/ml of steroid substrate (testosterone oenanthate, or hydroxy progesterone caproate)in 100-ml flask was inoculated with 100 µl of bacterial inoculum of approximately 10^8 CFU/ml froman overnight culture in Trypticase soy broth. Controls without substrate, uninoculated reaction mixture, and culture media were run in parallel with the test. All flasks were incubated with shaking at 220-250 rpm/min at 37°C in a shaking water bath for 72 hours (Austin and Ellis, 2003). Cultures were inspected for malodor production by sensory assessment and subjected to thin layer chromatography (TLC) and gas chromatography–mass spectrometry (GC/MS) analysis.

Screening for malodor producing isolates by sensory assessment

Assessment of malodor production from testosterone oenanthate as a steroidal substrate was based on the *in vitro* odor production model describedby Froebe *et al.,* (1990) and Rennie *et al.,* (1990) by smelling the odor of the blinded cultures and controls by a panel of two or three judges and each was given a description according to the presence and intensity of malodor produced.

Thin Layer Chromatography (TLC)

Test isolates' cultures containing steroid substrateas well as controls were extracted each with 2 X 5 ml ethyl acetate and the pooled extracts were evaporate and the residues were dissolved in 1 ml methanol. Five microliters of methanolic extracts were spotted onto silica gel plates that were developed using chloroform-methanol (95:5 v/v) solvent systems (Edwards *et al.,* 2003). The developed plates were examined under UV lamp (254 nm) (Petrino *et al.,* 1993), or after exposure to iodine vapours (Lupo Di Prisco*et al.,* 1968) to visualize the spots and retention values (Rf) were calculated for resolved spots.. Rf, is defined as the distance traveled by the compound divided by the distancetraveled by the solvent from the base line

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

Different solvent systems were used. Spots of the metabolites were interpreted comparing with references and controls.

Gas chromatography –mass spectrometric (gc/ms) analysis of metabolites

Ethyl acetate extracts of cultures and appropriate controls were evaporated in a rotary evaporator and the residues were dissolved in methyl alcohol.The crude extracts were first derivatized into t-butyldimethylsilyl ethers (TBDMS) to protect hydroxyl group and o-pentafluorbenzyloxime (PFB) to protect ketonic group (Gower *et al.,* 1997b; Austin and Ellis, 2003).The derivatized extracts were subjected to gas chromatography-mass spectrometry using a Shimadzu instrument (GC/MS QP5050A) fitted on DB-1 column (J&W Scientific) with a length of 30 m, inner diameter of 0.53 mm, and film thickness of 1.5 µm. The temperature program was initially set to 80°C for 2 min (10°C/min), then subsequently increased to 200°C (20°C/min) and finally held at 300°C for 1 min (injector temperature, 280°C;

detectortemperature, 300°C). Carrier gas was helium, ionization mode was at 70-eV, detector voltage was 2.74 kV, and the mass range was 40-550 *m/z*. Steroid metabolites were identified by prediction from molecular ion and fragmentation patterns.

Screening for potential inhibitors of malodor production

Some substances were tested for their malodour inhibiting potential. The same procedure for screening for malodour production mentioned above was used with the exception that the test substance was added, at sub-inhibitory concentration; were surface inoculated with 5 µl of the test isolates. A 0.5 McFarland standardized suspension of the test organism (equivalent to 1.5×10^8 cells/ml) prepared from a concentration that's equal or less than 1/4 MIC which was taken, as the lowest concentration of antimicrobial agents at which there was no visible growth of the organism.

Determination of bacterial inhibitory effect of deodorants

The minimum inhibitory concentrations (MICs) of the potential antimicrobial deodorant against test isolates were determined by agar dilution method according to NCCLS (2002). Two-fold serial dilutions of the tested compounds were prepared in Mueller-Hinton agar medium (Oxoid). The plates overnight culture in brain heart infusion broth was further diluted so as to contain approximately 10^5 CFU per spot. The plates were incubated at 37^{o}C for 18 h.

Table 1. Screening for malodor producing isolates from testosterone oenanthate by sensory assessment and TLC.

Species	No. of isolates	Odor description	Rfvalues for resolved spots
C. jeikeium	16	Urinous bad odor	0.4, 0.5, 0.6, 0.7, 0.9
C. macginleyi	4	Urinous bad odor	0.4,0.5,0.6, 0.7,0.9
C. callionae	3	Distinctive bad odor	0.7, 0.9
C. cystitidis	3	Distinctive bad odor	0.7, 0.9
C. afermentans	2	Distinctive bad odor	0.7,0.9
C. amycolatum	3	Distinctive bad odor	0.7,0.9
C. minutissimum	4	Distinctive bad odor	0.7,0.9
C. pilosum	3	Distinctive bad odor	0.7,0.9
C. striatum	3	Distinctive bad odor	0.7,0.9
C. xerosis	4	Distinctive bad odor	0.7,0.9
S. aureus	10	Moderate odor	0.7,0.9
S. epidermidis	13	Moderate odor	0.7,0.9
S. hominis	4	Moderate odor	0.7,0.9
S. cohnii	4	Moderate odor	0.7,0.9
S. capitis	3	Urinous bad odor	0.7,0.9
S. saprophyticus	5	Moderate odor	0.7,0.9
M. luteus	6	Rancid bad odor	0.7,0.9
M. lylae	4	Rancid bad odor	0.7,0.9
D. nishinomiyaensis	3	Rancid bad odor	0.7,0.9

RESULTS

One hundred and ninety isolates recovered from volunteers were identified to species level representing 111 *Staphylococcus spp*., 34 *Micrococcus spp*. and 45 *Corynebacterium spp*. All *Cornyebacterium* isolates and representative isolates of *Staphylococcus* (39) and *Micrococcus* (13) were tested for their ability to produce malodor from cultures containing testosterone ester as substrate.

Screening for malodor producing isolates from testosterone oenanthate

All tested *Micrococcus* isolates produced a bad rancid odor but not a sweaty ruinous odor. None of the tested staphylococcal isolates could produce distinctive malodor except *S. capitis* isolates (3) which produced a strong urinous malodor. All lipophilic corynebacteria (16 *C. jiekieum* and 4 *C. macginleyi*) produced offensive sweaty urinousodor, while non-lipophilic *Corynebacterium* species produced less intensive malodor. Control cultures produced no malodor.

TLC analysis for cultures of isolates demonstrating bad odor from testosterone revealed three extra spots,possibly representing the metabolites with Rf values 0.6, 0.5 and 0.4;in addition to two spots representing the substratecontrol with Rf values 0.9 and 0.7.Cultures of isolates that did not produce malodor from testosterone, showed no extra spots in addition to that of the substrate. Controlsof culture media and inoculated cultures without substrate showed no distinctive spots (Table 1). GC-MS analysis of extracts from culture producing malodor (Figures 1 & 2) showed two major bacterial metabolites of testosterone oenanthate (peaks 1 & 2).

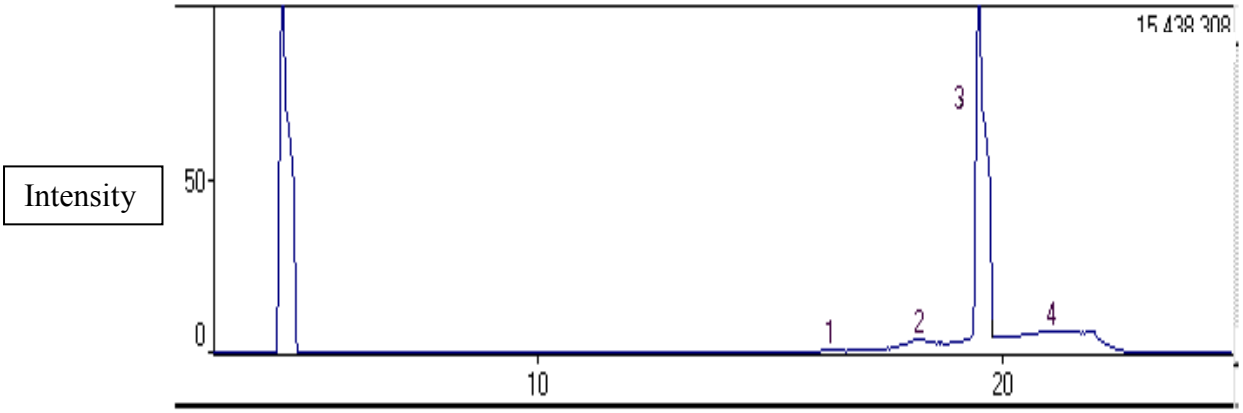

Figure 1. GC/MS analysis of extract of control containing culture media and testosterone oenanthate showing the substrate at peak 3.

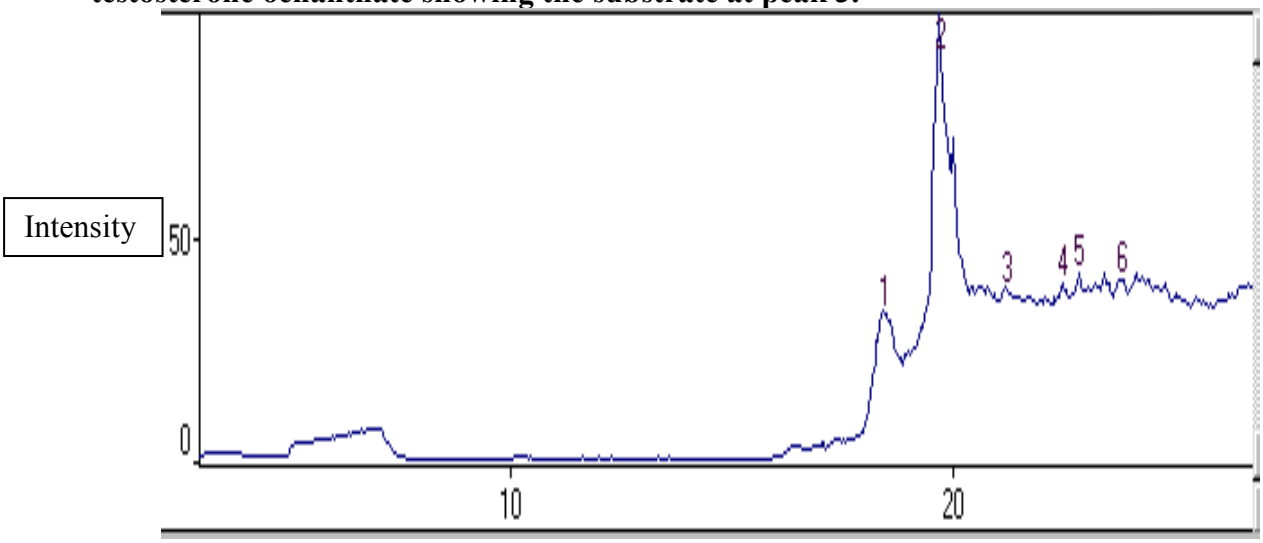

Figure 2. GC/MS analysis of extract of *Corynebacterium jeikeium* culture containing testosterone oenanthate after 72 hours incubation, showing two major metabolites of the testosterone oenanthate at peak 1 & 2.

Screening for malodor producing isolates from hydroxy progesterone caproate

Ethyl acetate extracts of inoculated reaction mixture and controls were subjected to TLC. The mobile phase used was chloroform: ethanol (9:1 v/v). It was found that only testosterone metabolizing isolates could metabolize hydroxy progesterone caproate and produce non-odorous metabolites. Lipophilic *Corynebacteria* and *Staphylococcus capitis* isolates were found to be able to produce two major non-odorous metabolites (I & II) from hydroxy progesterone caproate as the substrate with Rf values 0.9 and 0.5 in (chloroform :methanol). Metabolite I is more polar than the substrate with Rf value 0.43 and metabolite II which is less polar than the substrate with Rf value 0.58 (Table 2). Controls containing substrate showed the Rf values of 0.9 and 0.5. Controls containing microbiological media showed no spots.

Table 2. Screening for malodor producing isolates from hydroxyprogesterone caproate by sensory assessmentand TLC chloroform:methanol (95:5 v/v)

Species	No. of isolates	Odor description	Rf values for resolved spots
C. jeikeium	16	Faint bacterial culture odor	0.43, 0.5, 0.58, 0.9
C. macginleyi	4	Faint bacterial culture odor	0.43,0.5,0.58, 0.9
C. callionae	3	Faint bacterial culture odor	0.5, 0.9
C. cystitidis	3	Faint bacterial culture odor	0.5, 0.9
C. afermentans	2	Faint bacterial culture odor	0.5, 0.9
C. amycolatum	3	Faint bacterial culture odor	0.5, 0.9
C. minutissimum	4	Faint bacterial culture odor	0.5, 0.9
C. pilosum	3	Faint bacterial culture odor	0.5, 0.9
C. striatum	3	Faint bacterial culture odor	0.5, 0.9
C. xerosis	4	Faint bacterial culture odor	0.5, 0.9
S. aureus	10	Faint bacterial culture odor	0.5, 0.9
S. epidermidis	13	Faint bacterial culture odor	0.5, 0.9
S. hominis	4	Faint bacterial culture odor	0.5, 0.9
S. cohnii	4	Faint bacterial culture odor	0.5, 0.9
S. capitis	3	Faint bacterial culture odor	0.43, 0.5, 0.58, 0.9
S. saprophyticus	5	Faint bacterial culture odor	0.5,0.9
M. luteus	6	Rancid bad odor	0.5, 0.9
M.lylae	4	Rancid bad odor	0.5, 0.9
D. nishinomiyaensis	3	Rancid bad odor	0.5, 0.9

Screening for potential inhibitors of malodor production by sensory assessment and TLC

Test substances, at sub-inhibitory concentrations (≤ 1/4 MIC),were screened for inhibition of malodor production in *Corynebacterium jeikeium* cultures containing testosterone oenanthate after 72 hours incubation by sensory assessment.Extracts of cultures in presence and absence of inhibitors were analysed by TLC (Table 3 and Figure 6). Bergamot oil, vanillin and peppermint extract, masked the formed odor but did not inhibit metabolite formation. Ascorbic acid, retinyl palmitate, α-tocopherol acetate and selenium dioxide inhibited the formation of malodorous metabolites. Also, triethyl citrate, mastic gum, cupric sulfate, zinc sulfate, EDTA and orlistat inhibited malodor formation. Triclosan, ethylene glycol, polyethylene glycol, musk xylol and alum did not inhibit the formation of malodorous metabolites (Table 3). Extracts of the reaction mixture that demonstrated additional spots over the reaction mixture containing inhibitorwere subjected to further investigation.

Table 3.Evaluation of malodor inhibiting potential of tested material by sensory assessment and TLC

Material tested	Conc (%w/v)	Odor description**	TLC	
			Spots number	Rf
$ZnSO_4$	0.2	F	2	0.9, 0.5
$CuSO_4$	0.1	F	2	0.9, 0.5
Triethyl citrate	2	F	2	0.9, 0.5
Orlystat	(0.04)	F	2	0.9, 0.5
α-tocopherol acetate	2	F	3	0.9, 0.5,0.6*
Retinylpalmitate	2000 IU	F	3	0.9, 0.5,0.95*
Selenium dioxide	0.0025	F	2	0.9, 0.5
Ascorbic acid	0.05 (0.006)	F	2	0.9, 0.5
Vanillin	0.0025	M	6	0.9, 0.5, 0.45, 0.4, 0.35, 0.2*
Mastic gum	0.025 (0.006)	M	3	0.9, 0.5, 0.45
EDTA	0.025	M	2	0.9, 0.5
Bergamot oil	0.1	MB	5	0.9,0.5, 0.45, 0.4, 0.35
Peppermint oil	0.016	MB	5	0.9,0.5, 0.45, 0.4, 0.35
Alum	0.1	USB	5	0.9,0.5, 0.45, 0.4, 0.35
Ethylene glycol	0.2	USB	5	0.9,0.5, 0.45, 0.4, 0.35
Triclosan	0.00005	USB	5	0.9,0.5, 0.45, 0.4, 0.35
Polyethylene glycol	1	USB	5	0.9,0.5, 0.45, 0.4, 0.35
Musk Xylol	1	USB	5	0.9,0.5, 0.45, 0.4, 0.35

*: Additional spot
** F= faint odor; M= moderate odor; MB= masked bad odor; USB= uruinous sweaty bad odor

Table 4. GC/MS data of the testosterone oenanthate metabolites by *Corynebacterium jeikeium* and the possible metabolites identity.

Substance	Retention time	Mass peak	Molecular Weight	Possible Molecular formula	Suggested possible compound	%
Substrate	19.6	114	400	$C_{26}H_{40}O_3$	Testosterone oenanthate	100
Metabolite I	18.47	237	270	$C_{19}H_{26}O$	Androstadienone	22.3
Metabolite II	19.7	287	290	$C_{19}H_{30}O_2$	5-α-dihydrotestosterone	75.3

Assessment of inhibition of malodor production by gas chromatography mass spectrometry (GC/MS)

The effect of malodor production inhibitors on conversion of substrates into metabolites by the action of *C. jeikeium*was quantitatively assessed after 72 hours using GC/MS (Table 5 and Figures 3, 4&5). In the absence of inhibitor (Figure 2), 99.6% oftestosterone ester substrate was converted into metabolites. In the presence of mastic gum (Figure 3), about 84.27% of the substrate was converted into metabolites. In the presence of ascorbic acid (Figure 4), only about 6.9 % of the substrate was converted into metabolites. While in the presence of orlistat (Figure 5), about 0.6 % of the substrate was converted into metabolites.

Screening for malodor producing isolates from hydroxy progesterone caproate

Ethyl acetate extracts of inoculated reaction mixture and controls were subjected to TLC. The mobile phase used was chloroform: ethanol (9:1 v/v) .It was found that only testosterone metabolizing isolates could metabolize hydroxy progesterone caproate and produce non-odorous metabolites.Lipophilic *Corynebacteria*and *Staphylococcus capitis*isolates were found to be able to produce two major non-odorous metabolites (I & II) from hydroxy progesterone caproate as the substrate with Rf values 0.9 and 0.5 in (chloroform :methanol). Metabolite I is more polar than the substrate with Rf value 0.43 and

metabolite II which is less polar than the substrate with Rf value 0.58 (Table 2). Controls containing substrate showed the Rf values of 0.9 and 0.5. Controls containing microbiological media showed no spots.

Table 5. Quantitative assessment of inhibitors of malodor formation using GC/MS.

Inhibitor of malodor	Concentration of inhibitors (mg/ml)	% of converted substrate into metabolites	% of unconverted substrate	Retention time of the substrate.	Peak number
In absence of inhibitor	_	99.6	0.44	21.2	3
Orlistat	1.5	0.6	99.41	21.4	5
Ascorbic acid	0.25	6.9	93.02	20.24	5
Mastic gum	0.25	84.27	15.73	20.6	11

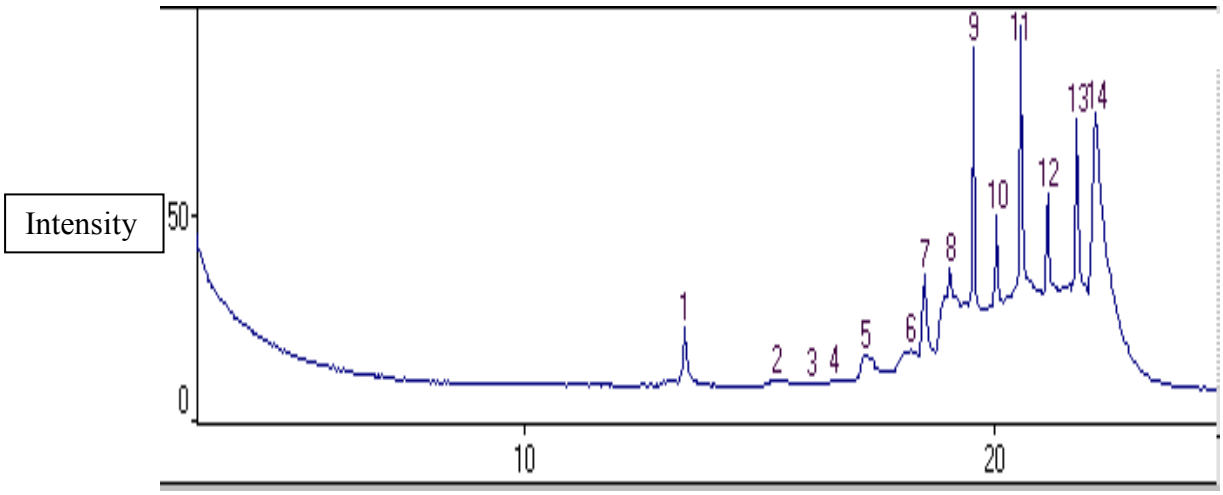

Figure 3. GC/MS analysis of the extract of *C. jeikeium* culture containing testosterone oenanthate as substrate in the presence of mastic gum after 72 hours incubation, showingabout 84.27% of the substrate was converted into non odorous metabolites.

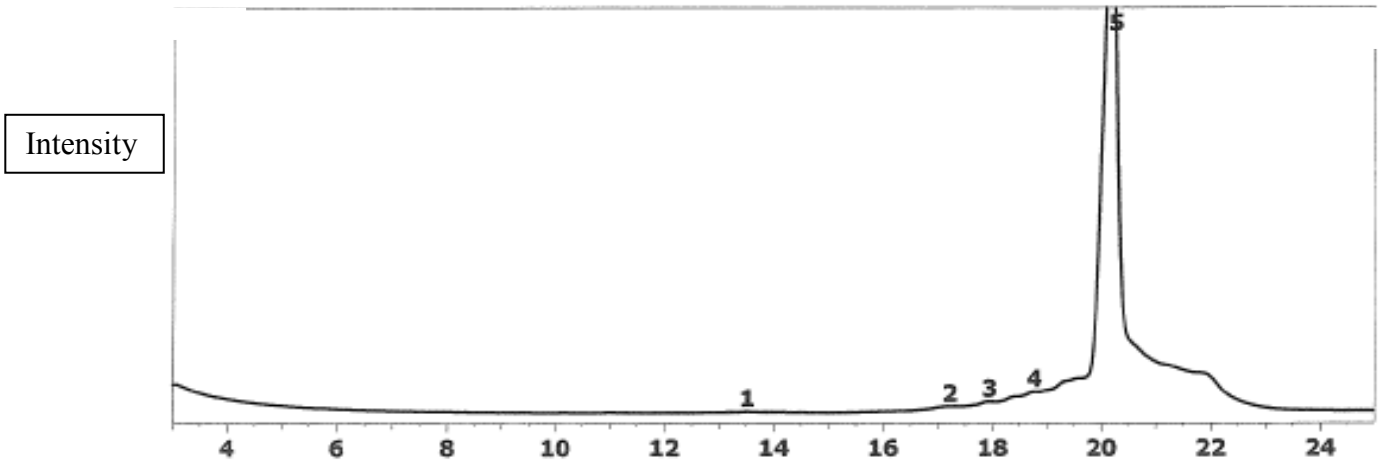

Figure 4. GC/MS analysis of the extract of *C. jeikeium* culture containing testosterone oenanthate as substrate in the presence of ascorbic acid after 72 hours incubation, showing only about 6.9 % of the substrate was converted into metabolites.

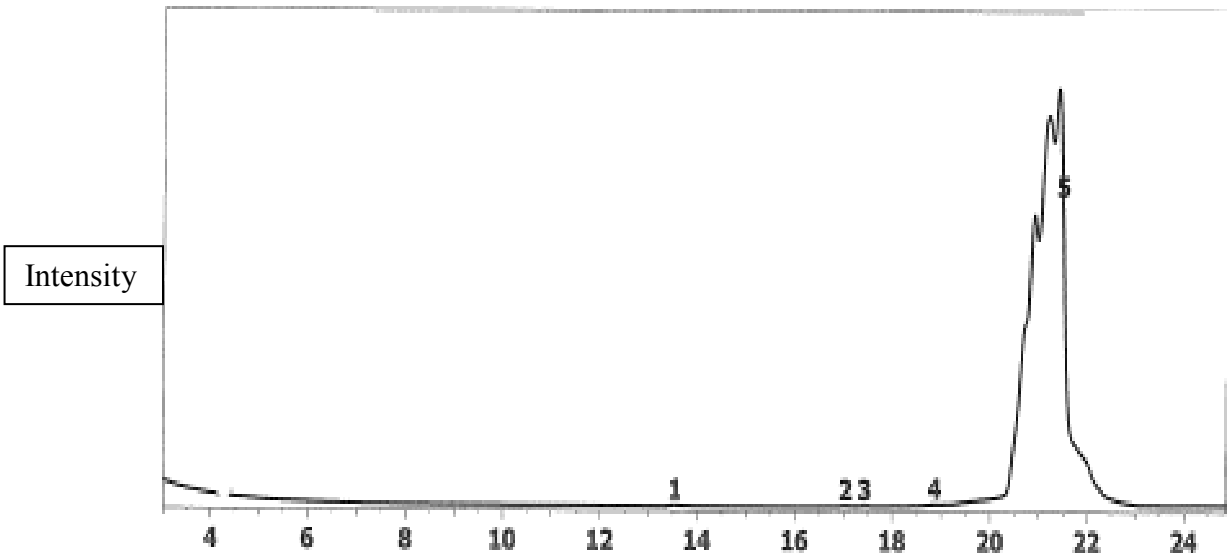

Figure 5. GC/MS analysis of the extract of *C. jeikeium*culture containing testosterone oenanthate as substrate in the presence of orlistat after 72 hours incubation, showing only about 0.6 % of the substrate was converted into metabolites.

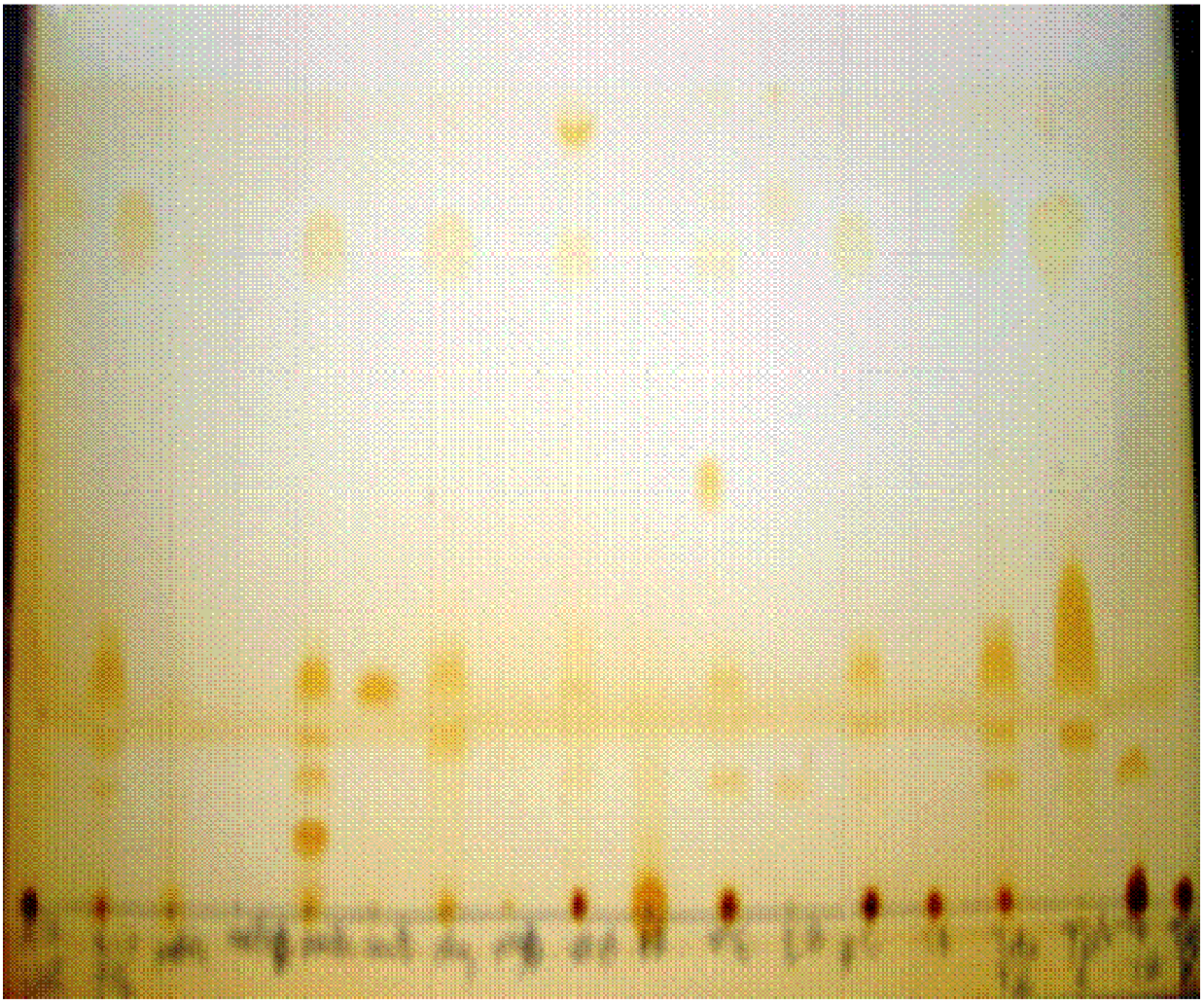

Figure6. Thin layer chromatography of culture extracts of *C. jeikeium* in the presence and absence of various inhibitorsafter 72 hrs incubation. Lane 1: reaction mixture at zero time; Lane 2: reaction mixture after incubation; Lane 3 reaction mixture after incubation with mastic gum; Lane 4, mastic gum control; Lane 5, reaction mixture after incubation with vanillin; Lane 6, vanillin control; Lane 7, reaction mixtureafter incubation with orlystat; Lane 8, orlystatcontrol; Lane 9, reaction mixture after incubation with retinylpalmitate; lane 10, retinylpalmitatecontrol; Lane 11, reaction mixture after incubation with α-tocopherol acetate; Lane 12, α-tocopherol acetatecontrol ; Lane 13, reaction mixture after incubation with ascorbic acid; lane 14, ascorbic acid control; lane 15, reaction mixture after incubation without Tween 80; Lane 16, testosteroneoenanthatecontrol; Lane 17, reaction mixture after incubation without testosterone; Lane 18, Tween 80 blank.

DISCUSSION

The generation of malodor on various sites of the human body is caused by the microbial biotransformation of odorless natural secretions into volatile odorous molecules. On the skin surface, distinctive odors emanate, in particular, from the axilla, where a large and permanent population of microorganisms thrives on secretions from the eccrine, apocrine, apoeccrine and sebaceous glands (Leyden *et al.,* 1981). Axillary odor is due to a large number of odorous substances, including aliphatic fatty acids, 3M2H acids, odorous androst-16-ene steroids, as well as the 2-ene steroid (Gower *et al.,* 1997).

The most fundamental approach to deodorancy is to prevent the formation of these odorous compounds, the bacteria present in the axilla are largely responsible

for the biotransformations leading to odorous compounds, and consequently the earliest and most widespread strategy aimed at the inhibition of odor formation has involved the use of antimicrobials (Woodruff, 1994).

The control of malodor is achieved by various mechanisms:the use of antimicrobial agents, pH modification, enzyme inhibitors (esterase), and inhibition of odorous steroid formation, inhibition of volatile fatty acid formation, inhibition of protein and amino acid biotransformation, inhibition of bacterial adherence, odor counter-actants,and fragrances and modifiers of perception.

In the present study, bacterial isolates were recovered from armpit samples taken from individuals with distinctive production of malodor. The isolates obtained belonged to the most commonly isolated genera from axilla (Marples and Williamson, 1969; Kloos and Mussolwhite, 1975; Leyden *et al.,* 1981; Jackman and Noble, 1983; Cox, 1987).The isolates were screened for their ability to produce malodorous metabolites from testosterone oenanthate ester. Malodor producing isolates were detected by sensory assessment and thin layer chromatography (TLC). Except for *S. capitis* isolates that produced urinous bad odor, there was no distinctive malodor produced by *Staphylococcus* isolates. This is the first time to report malodor production by *Staphylococcus capitis* from testosterone. All *Micrococcus* isolates produced a bad rancid odor, but not a sweaty urinous odor.

The tested isolates of lipophilic species of *Corynebacterium (C. jiekieum* and *C. macginleyi*) produced the most offensive sweaty urinous odor, concurrently with the metabolic transformation of testosterone oenanthate, as demonstrated by TLC, whereas other non lipophilic *Corynebacterium* species produced a less distinctive odor. Lipophilic *Corynebacterium* isolates were reported to produce malodorous metabolites from testosterone (Leyden *et al.,* 1981; Jackman and Noble, 1983; Gower *et al.,* 1985; Nixon *et al.,* 1986 & 1987; Froeb *et al.,* 1990; and Rennie *et al.,* 1990).

Using hydroxy progesterone caproate as a substrate instead of testosterone, all *Micrococcus* isolates showed no distinctive metabolites spots, but exhibited a bad rancid odor. In agreement with these findings, Decr´eau*et al.* (2003) found that *Micrococcus* spp. did not metabolize sterols. On the other hand, all tested *Corynebacterium* isolates showed distinctive metabolites spots but did not exhibit distinctive malodor. In accordance with this, no malodorous metabolites were obtained from pregnenolone which is another C21 putative female hormone (Austin and Ellis, 2003).

GC/MS was used for quantification of testosterone ester and identification of its metabolites by comparing the molecular ions formed and fragmentation patterns, retention time, molecular weight with previously reported data. Two major metabolites were found. Predictions were made from molecular ions formed and different fragmentation patterns. Metabolite I (22.3% of total metabolites) would match androstadienone (Labows, 1988) which is known to have urinous odor (Gower *et al.*, 1997b. Labows, 1988). The second metabolite II (75.34% of total metabolites) could be 5-α-dihydrotestosterone (Nixon *et al.,* 1984) which is not known to exhibit odor. When compared with the previously reported data (Nixon *et al.,* 1984), and supposed molecular formula would suggest that similarly (Table 1). Androstadienone could be formed by the dehydration at the 17-hydroxy group and the formation of a double bound at C-16 (Gower *et al.*, 1997b), whereas 5α-dihydrotestosteroneis formed by reduction of the double bond of testosterone (Nixon *et al.,* 1984).

Rennie *et al.,* (1990) have found that many aerobic coryneforms could

transform testosterone, the principal metabolites being 5α- and 5-β-di-hydrotestosterone, androstenedione, and 5-α- and 5-β-androstanedione.

In the present study, some components of deodorant products and raw natural products material or chemical with potential deodorant activities were tested for their ability to prevent malodorous metabolites formation from testosterone oenanthate. Materials with known antimicrobial activities were used at sub-inhibitory concentrations to exclude their effect as inhibitors of microbial proliferation as a mechanism of deodorant activities.

Cupric sulfate, zinc sulfate, triethyl citrate, orlistatselenium dioxide, ascorbic acid and EDTA were found to inhibit malodor production from testosterone ester without the appearance of extra spots by TLC. In agreement with these finding, Froeb*et al.,* (1990) reported thatcupric sulfate, zinc sulfate, di-sodium EDTA inhibit malodor forming metabolic pathway. α-tocopherol acetate, and retinyl palmitate reduced the odor intensity but produced one extra spot with TLC, these were found to be attributed to interaction with the filtrate of the test culture. The fact that the steroidal metabolism in bacteria is accelerated by a number of oxido-reductase enzymes as 5-α-reductase, 3-hydroxy steroid dehydrogenase, 1-ene reductase, 4-ene reductase and other enzymes, could explain the present and the previously reported findings (Decr´eau *et al.,* 2003; Gower *et al.,* 1988; Nixon *et al.,* 1986) that some antioxidants were able to inhibit the possible malodorous metabolites.

Vanillin, Mastic gum, bergamot oil, peppermint oil, alum, ethyleneglycol, polyethylene glycol, triclosan, alum and musk xylol could not inhibit the metabolism of testosterone ester. The fragrant components, namely vanillin, peppermint oil and bergamot oils, seem to have worked through masking the bad odor. Mastic gum seemed to have sequestered the metabolite preventing its extraction, as appeared in TLC, or it could have led to the production of other metabolites of less intensive odor as appeared in its GC/MS charts. The other components could not inhibit the urinous sweaty odor at the tested concentrations, such compounds possibly owes its deodorant action through their antimicrobial activities as the case with glycols and triclosan (Fearnley and Cox, 1983; Cox, 1987; Block, 1991) or through their antiperspirant activities as with alum (Quatral*et al.,* 1997; Piérard*et al.,* 1993).

Triethyl citrate was proposed to act as deodorant through preferential hydrolysis (competitive inhibition), by esterases produced by sweat metabolizing bacteria (Osberghaus, 1980).

The present findings demonstrate that vanillin, peppermint and bergamot oils masked the formed odor but did not inhibit metabolite formation.

Orlistat (tetrahydrolipstatin) is a semisynthetic derivative of naturally occurring lipase inhibitor (lipase is specific estrase) was found to inhibit malodor formation from testosterone onenthateby *Corynebacteria*. Here it was shown for the first time, that orlistat was able to inhibit malodor production. A number of non-antibacterial esterase inhibitors have been patented for use in deodorants (Maurer *et al.,* 1995; Wachter *et al.,* 1995).

GC/MS analysis revealed almost complete (99.6%) conversion of testosterone ester into metabolites in *C. jeikeium* culture after 72 hour incubation. In the presence of mastic gum, about 84.3% of the substrate was converted into metabolites, yet no malodor was found, indicating that mastics inhibited the malodor associated metabolism possible by directly interacting with and sequestering the substrate or metabolites (as TLC show only scanty faint spots of the substrate) or through diverting the metabolic reaction to different pathways leading to the formation of odorless metabolites or the formation of

malodorous metabolites in very low quantity. While ascorbic acid dramatically inhibited testosterone metabolism (6.9 % of the substrate was converted into metabolites), orlistat almost completely prevented its metabolism (only 0.6 % of the substrate was converted into metabolites).

The present findings demonstrate the high potentials of orlistat, ascorbic acid and mastic gum as deodorants. Further work is required to define their mode of action and test their applications as deodorant ingredients.

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تقييم خارج الجسم لفعالية بعض مكونات المستحضرات المزيلة لرائحة العرق وتقييم قدرة كل من الاورليستات وحمض الاسكوربيك و صمغ المستكه على تثبيط المسار الأيضي المكون للرائحة الكريهة

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تلعب البكتريا الابطية الطبيعية دورا في تكوين رائحة العرق الكريهة عن طريق تحلل السلائف عديمة الرائحة لهذه المركبات الناتجة الي مركبات كريهة الرائحة. تم عزل ١٩٠عزلة من ١٠٠مئة متبرع يشترط ان يكونوا اصحاء ولا يتستخدمون اي علاج ولم يستعملوا اي مزيلات عرق. و وجد ان هذه العزلات تتكون من ١١١ من المكورات العنقودية و٣٤ من المكورات الدقيقة و ٤٥ من البكتيريا الوتدية.

تم اختبارقدرة جميع العزلات على تكوين مستقلبات كريهة الرائحة من استراوننثات هرمون التستوستيرون بواسطة التقييم الحسي وبواسطة تقييم الفصل اللوني. وجد أنه فقط سلالات البكتيريا الوتدية المحبة للمواد الدهنية والمكورات العنقودية الرأسية هي القادرة على تكوين المستقلبات كريهة الرائحة. تم اختبار قدرة العزلات على تكوين مستقلبات كريهة الرائحة من استركبروات هرمون البروجستيرون و وجد انه فقط السلالات القادرة على تأيض إستر هرمون تستوستيرون هي القادرة على تأيض إستر بروجستيرون هيدروكسي ونتج عن هذا التفاعل الأيضي مستقلبات عديمة الرائحة.

تم تحليل المستقلبات كريهة الرائحة للتستوستيرون والتعرف عليها باستخدام الفصل الغازي تحليل الطيف الكمي. وجد مستقلبين رئيسيين هما اندروستاديينون و ثنائي هيدروجين التستوستيرون .

تم اجراء تقييم كمي لتحول استراوننثات التستوستيرونالي مستقلبات بواسطة البكتيريا الوتدية في وجود كل من الاورليستات وحمض الاسكوربيك و صمغ المستكه بواسطة الفصل الغازي و التحليل الطيف (GC-MS). وجد أنه تم تحول نسبة كبيرة من المادة الاولية من استراوننثات التستوستيرون في عدم وجود مواد مثبطة (٩٩,٦٪). أما مع وجود كل من الاورليستات تم تحول (٠,٦٪) وحمض الاسكوربيك تم تحول (٦,٩٪) مما يدل على قدرة كلاهما على تثبيط المسار الأيضي المكون للرائحة الكريهة. أما في وجود صمغ المستكه فقد تم تحول (٨٤,٢٧٪) وتوصي هذه الدراسة باستخدام هذه المواد أو اضافتها كمكونات أساسية لمزيلات رائحة العرق.