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Research Article

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Microbial Transformation of 3,3-Dimethylcyclohexyl methyl ketone and Antimicrobial Evaluation

3,3-Dimetilsikloheksil metil ketonun Mikrobiyal Dönüşümü ve Antimikrobiyal Değerlendirmesi

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ABSTRACT

3,3-Dimethylcyclohexyl methyl ketone (Herbac®) is a fine fragrance compound used in the cosmetic industry. In this study, the biological derivatisation of the substrate 3,3-dimethylcyclohexyl methyl ketone by 18 fungal cultures was carried out to obtain new derivatives. Among the evaluated, the plant fungus *Aspergillus niger* ATCC 10549, biotransformed the substrate to the 4-hydroxy derivative in 19% yield. The structure was determined by using NMR and GC-MS methods. In addition, the antimicrobial activity of Herbac® with the metabolite against a panel of pathogenic microbial strains were evaluated using an *in vitro* microdilution assay. The results showed that the metabolite was relatively more susceptible to the pathogens compared to the substrate

Key Words

Antimicrobial activity; *Aspergillus niger*; biotransformation; fragrance compounds.

ÖZ

3,3-Dimetilsikloheksil metil keton (Herbac®), kozmetik endüstrisinde kullanılan karakteristik bir koku bileşiğidir. Bu çalışmada, yeni türevler elde etmek için 3,3-dimetilsikloheksil metil keton substratının 18 fungus kültürü ile biyolojik türevlendirilmesi gerçekleştirilmiştir. Değerlendirilenler arasında, bitki kökenli *Aspergillus niger* ATCC 10549, substratı %19 verimle 4-hidroksi türevine biyolojik olarak dönüştürdü. Yapı, NMR ve GC-MS spektroskopik metodlar kullanılarak aydınlatıldı. Ek olarak, substrat ile metabolitinin patojenik mikrobiyal suşlara karşı antimikrobiyal aktivitesi, *in vitro* mikrodilüsyon kullanılarak değerlendirildi. Sonuçlar metabolitin patojen mikroorganizmalara karşı substratına kıyasla nispeten daha duyarlı olduğunu göstermiştir.

Anahtar Kelimeler

Antimikrobiyal aktivite; *Aspergillus niger*; biyotransformasyon; koku bileşikleri.

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INTRODUCTION

Flavour and fragrance compounds are utilized in food, cosmetic, chemical and pharmaceutical industries among others [1, 2]. Natural product based flavour and fragrance comounds gained more attention for the development of eco-friendly industrial processes and end products. Biotechnological processes, especially biotransformation is accepted as a natural way, for the production of fragrance compounds, which is among the options in this context. In general, still large scale production of fragrance materials rely mainly to synthetic routes [3]. Musk odorants, described as sensual and natural, are known to constitute important classes of fragrances, which are versatile odorants and raw materials used in perfumery. As it is wellknown, synthetic musks are a class of compounds to emulate the scent of deer musk and other historically animal originated musks [4].

The carotenoid derivative, 3,3-dimethylcyclohexyl methyl ketone, having a geminal dimethyl-substituted ring function group, is classified among the musk odorants, which is commercially produced and used as Herbac® by International Flavors and Fragrances - IFF Inc. Company. Such compounds are mostly combined to fine fragrances, body lotions, other and toiletries, however also in household products such as detergents and other specific consumer products [5-7].

In the continuation of our research on functional flavour and fragrance compounds, the present study aimed the production of new derivatives from 3,3-dimethylcyclohexyl methyl ketone, by initial microbial transformation screens using 18 different fungi. In addtition, *in vitro* antimicrobial activity asssays were performed to compare the substrate and the metabolite activity. To the best of our knowledge, this is the first report on the microbial transformation of 3,3-dimethylcyclohexyl methyl ketone and biological evaluation.

MATERIALS and METHODS

General experimental procedures

The substrate 3,3-dimethylcyclohexyl methyl ketone - Herbac® was kindly provided by IFF Inc. Co., USA. Furrier Transform Infra Red (FTIR) spectra were obtained using KBr pellets on Bruker Tensor 27 Spectrometer. Optical rotations were recorded using the Krüss Optronic P8000-T polarimeter. Agilent 6890N GC system and GC/MS Agilent 5975 GC/MSD, Shimadzu QP2010 Plus with innowax FSC column (60 m × 0.25 mm and 0.25 µm film thickness) and CPSil-5CB (25 m × 0.25 mm and 0.25 µm film thickness) were used. High resolution mass spectra (HRMS) were analysed at electrospray ionization (ESI) with micro-TOF. NMR spectra were aqurired by Varian Mercury Plus 400 in deuterio chloroform ($CDCl_3$). Silica gel (230–400 mesh, type 60) was used for column chromatography purifications. Thin Layer Chromatography (silica gel 60 GF_{254}) was performed using light petroleum/ethyl acetate in various proportions, where the metabolites were spotted by UV light and by acid staining followed by heating.

Microorganisms

All microbial strains were provided from the Agriculture Research Service Culture Collection (NRRL), American Type Culture Collection (ATCC) and the culture collection of the Faculty of Pharmacy at Anadolu University, Turkey.

Microbial strains used for biotransformations were namely; *Aspergillus parasiticus* NRRL 2999, *A. niger* ATCC 10549, *A. niger* NRRL 326, *A. alliaceus* NRRL 317, *Penicillium claviforme* MR 376, *P. adametzii* NRRL 737, *P. chrysogenum* NRRL 792, *Fusarium solani* ATCC 1284, *F. moniliforme* NRRL 2374, *F. culmorum* isolate (Faculty of Sciences), *Hansenula anomala* ATCC 20170, *Mucor ramannianus* ATCC 1839, *Neurospora crassa* (wild type), *Sporobolomyces pararoseus* ATCC 11385, *Saccharomyces cerevisiae* ATCC 9763, *Trametes versicolor* ATCC 200801, *Corynespora cassiicola* DSM 62475 and *Phanerachaete chrysosporium* ME (isolate), respectively.

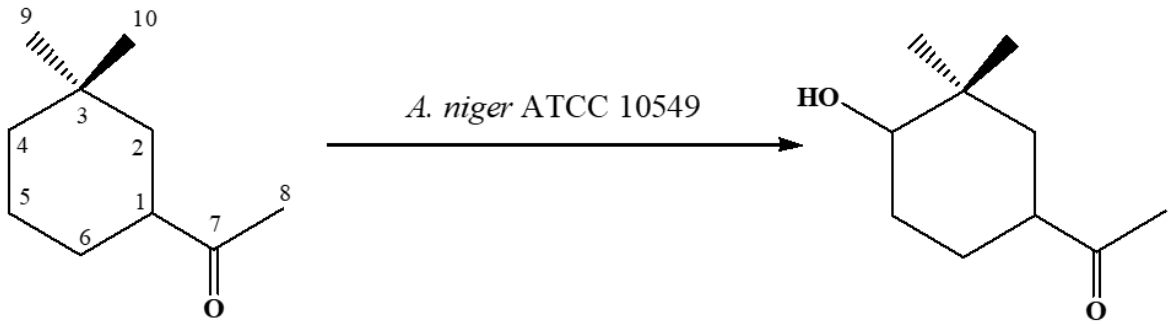
Strains used for *in vitro* antimicrobial evaluation: The human pathogenic strains were *Staphylococcus epidermidis* ATCC 12228, *Proteus vulgaris* NRRL B-123, *Pseudomonas aeruginosa* ATCC 27853, Meth. Resist. *S. aureus* (MRSA clinical isolate), *Escherichia coli* NRRL B-3008 and *Corynebacterium* sp. (14A), and *Candida parapsilosis* NRRL Y-12696.

Conditions of cultivation and biotransformation

The biotransformation experiments were basically conducted using the α-Medium as perviously described in detail [5-8].

Biotransformation by *A. niger*

3,3-Dimethylcyclohexyl methyl ketone substrate was transformed using the ATCC 10549 the strain over 7 days at 25°C to the metabolite in 19% yield, as shown in Scheme 1. The metabolite was purified as an oily odourus compound [25% ethyl acetate (v/v) in light petroleum]. This metabolite was assinged as 1-(4-hydroxy-3,3-dimethylcyclohexyl) ethanone based on FT-IR, HRMS and NMR data.



Scheme 1. Biotransformation of Herbac® by *Aspergillus niger.*

Table 1. Figure-of-merit (FOM) calculation of the sensor at different glycerol concentrations (FWHM represents full width at half maximum of the curves).

δC (ppm) for compounds		
C-position	Herbac®	Metabolite
C-1	47.5	46.7
C-2	40.9	41.0
C-3	38.5	35.5
C-4	30.4	76.9
C-5	21.5	29.8
C-6	28.1	27.2
C-7	211.9	211.7
C-8	27.8	28.2
C-9	24.3	17.9
C-10	33.0	28.9

$[\alpha]_D^{20}$: +78.68 (*c* 6.1, Acetone). FT-IR ν_{max} cm^{-1}: 3435 (OH), 1703 (C=O), 1261 (C-CH_3), 1064 (C-O). ^{1}H NMR ($CDCl_3$, 400 MHz): 0.92 (3H, s, 9-CH_3), 1.03 (3H, s, 10-CH_3), 1.22 (1H, t, *J* = 13.2 Hz, 2-H_{ax}), 1.35 (1H, m, 2-H_{eq}), 1.42 (1H, m, 5-H_{ax}), 1.59 (1H, m, 6-H_{ax}), 1.78 (1H, m, 5-H_{eq}), 1.88 (1H, m, 6-H_{eq}), 2.14 (3H, s, 8-CH_3), 2.48 (1H, m, 1-CH), 3.29 (1H, m, 4-CHOH). ^{13}C NMR: Table 1. HRMS $[M+Br]^+$ *m/z* 249.0858 (calcd for $C_{10}H_{18}O_2Br$, 249.0490).

In vitro antimicrobial evaluation

The antimicrobial evaluation of 3,3-dimethylcyclohexyl methyl ketone and its metabolite were tested by using the agar diffusion method, and the broth microdilition method for the determinaton of the minimal inhibitory concentration (MIC) of the new methyl ketone [9-11] as described in detail previous [12]. Anticrobial test were repeated in triplicates.

RESULTS and DISCUSSION

Among the used and tested 18 different fungal cultures, the biotransformation by *A. niger* 10549 was the most successful microorganism reported in this study. The molecular ion of the metabolite's MS data is *m/z* 170.25 representing $C_{10}H_{18}O_2$. The FTIR spectrum indicated a broad hydroxyl group absorption at 3435 cm^{-1}. The ^{1}H-NMR spectrum highlighted the proton signal at 3.29 ppm (1H, m, 4-CHOH) indicating that a new hydroxyl group was introduced at the 3,3-dimethylcyclohexyl methyl ketone structure. The ^{13}C-NMR spectrum confirmed this finding by showing the presence of a new hydroxyl-bearing signal at δC 76.8 ppm, whereas an associated signal resonating at 30.4 ppm disappeared simultaneously. The new position of the hydroxyl group was determined by the aid of HSQC and HMBC experimental data, which indicated the key connectivity of proton signals at 0.92 (3H, s, 9-CH_3), and 1.03 (3H, s, 10-CH_3) to the signals at δC 76.9 (C-4), respectively. These findings confirmed that the hydroxylation was at C-4 position. Interestingly, the metabolite was also reported as a microbial transformation product of 1-(3,3-dimethylcyclohexyl)ethanol [=cyclademol] by another *A. niger* NRRL 326 strain [13].

The *in vitro* antimicrobial activity of methyl ketone, and its aromatic hydroxy derivative were evaluated initially using an *in vitro* agar diffusion test at 4 mg/mL concentration, where the microbial inhibitions were observed against *Staphylococcus epidermidis,* Methycilline Resistant *S. aureus,* and the human pathogenic yeast *Candida parapsilosis.* The minimum inhibitory concentrations (MICs) for both compounds were obtained thereafter by the microdilution tests compared to standard antimicrobial reference substances, as shown in Table 2. The results indicated that the metabolite showed relatively higher antimicrobial activity compared to substrate against all microorganisms at the tested concentrations.

Table 2. Antimicrobial evaluation (MICs, mg/mL) data for herbac® and its metabolite

Microorganisms	Substrate	Metabolite	Chlorampenicol
Gram negative bacteria			
E. coli NRRL B-3008	2	0.5	0.0625
P. aeruginosa ATCC 27853	2	0.5	0.25
P. vulgaris NRRL B-123	2	0.5	0.03125
Gram positive bacteria			
S. epidermidis ATCC 12228	2	0.25	0.0156
MRSA (Clinical isolate)	2	0.25	0.0156
Corynebacterium sp. 14A	2	0.5	0.0156
Fungus			Ketoconazole
C. parapsilosis NRRL Y-12696	2	0.25	0.125

Conclusion

This study revealed new findings for the biotransformation and antimicrobial activity of the substrate 3,3-dimethylcyclohexyl methyl ketone and its aromatic metabolite. Also, the mono-hydroxylated derivative was more active compared to the starting material, the substrate indicating that biotransformations has functionalized the frangrant material contributing to its new antimicrobial features.

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