Evaluation of *in vitro* and *in vivo* antimicrobial and antifungal activity of Camelyn M product

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The minimum inhibitory concentration (MIC) of the active compound of the Camelyn M product obtained from a special type of honey in one region of Georgia was determined against certain strains of bacteria and fungi, both by agar and broth dilution methods. The antimicrobial activity of the Camelyn M product was then tested in animal models. Camelyn M was found to exhibit potent inhibitory activity (0.012-0.150 μg/ml) against most of the bacteria tested *in vitro*. *In vivo* studies showed that the drug provided significant protection ($p<0.001$) to mice exposed to the virulent bacterium.

In vitro, Camelyn M showed strong activity against fluconazole-resistant strains of *Candida albicans*, *Candida glabrata, Candida tropicalis, Candida parapsilosis* and *Candida krusei*, and the MIC value for inhibition of 90% of isolates was 0.012 μg/ml.

Keywords: Camelyn M, honey, antimicrobial, Candida, MIC.

Introduction

Antibiotics are one of our most important weapons in the fight against bacterial and fungal infections, and since their introduction they have contributed greatly to improving the quality of human life. However, over the past few decades, these health benefits have been threatened as numerous commonly used antibiotics have become increasingly ineffective in treating certain diseases, not only because many of them cause toxic reactions, but also because of the emergence of drug-resistant bacteria and fungi (2,3,4,5,6,7,8).

The ingredients in Camelyn M were isolated from special grades of honey from a region in Georgia (1).

Camelyn M is a mixture of various biologically active compounds: aldehydes, ketones and bioorganic acids (6). Various physicochemical parameters of the compounds contained in Camelyn M, such as solubility, primary structure, molecular weight and other properties, play an important role in the biological activity of this product
(6). Our studies have shown that Camelyn M has strong antibacterial and antifungal activity against a wide variety of bacterial and fungal species. Camelyn M acts as a detergent on the cell membranes of bacteria and fungi and selectively inhibits the vital mechanisms of bacterial and fungal cells.

This paper details the *in vitro* antibacterial and antifungal activity of Camelyn M
and *in vivo.*

Material and methodology

Media

The liquid media used in this study were peptone water [PW; Oxoid brand bacteriological peptone 1% [w/v] plus Analar NaCl 0.5% [w/v]), nutrient broth (NB; Oxoid), Mueller Hinton broth (MHB; Difco). The solid media are peptone agar (PA), bouillon agar with lactose and Chinese blue (BLA), nutrient agar (NA), and Mueller Hinton agar (MHA), obtained by solidifying the liquid medium with 1.2% (w/v) agar (Oxoid No. 3).

Antibacterial and antifungal preparations. Camelyn M is a honey-based product whose components were isolated from honey for *in vitro* and *in vivo* studies according to a previously described method (1). For *in vitro* studies, fluconazole (FLC) and itraconazole (ITC) were extracted from commercial preparations purchased from PSP Pharmaceuticals, Inc. (Tbilisi, Georgia).

Organisms:

Bacteria and Fungi: All bacterial and fungal strains were obtained from the American Biological Resource Center - American Type Culture Collection. Media

The liquid media used in this study were peptone water [PW; Oxoid brand bacteriological peptone 1% [w/v] plus Analar NaCl 0.5% [w/v]), nutrient broth (NB; Oxoid), Mueller Hinton broth (MHB; Difco). Solid media are peptone agar (PA), bouillon agar with lactose and Chinese blue (BLA), nutrient agar (NA), and Mueller Hinton agar (MHA), obtained by solidifying the liquid medium with 1.2% (w/v) agar (Oxoid No. 3). In the case of BLA.

In vitro studies The MIC value for the test organisms was determined using the broth microdilution method described in NCCLS document M27-A2 (3,4,5). The MIC value for Camelyn M was defined as the lowest concentration that caused little or no growth (inhibition of approximately 90%) after 48 h.

In vivo studies

A male Swiss strain of white mice weighing 18-20 g was used for *in vivo* testing. The virulence of the tested strain *S. typhimurium* NCTC 74 was amplified by repeated passaging in mice, and the median lethal dose (MLD or LD50) of the passaged strain corresponding to 0.95 x 10^9CFU/mouse (bacteria suspended in 0.5 ml NB) served as the challenge dose17 for all groups of animals. To ensure reproducibility of the provocative dose, its optical density was standardized in a Klett-Summerson colorimeter at 640 nm and the number of CFU in NA was determined.

To determine the toxicity of Camelyn M, 40 mice were used, 20 of which were injected with 60 g of the drug, and the remaining 20 received 30 g of Camelyn M. The mice remained under observation for up to 100 hours. Two groups of mice, 20 animals per group (each mouse weighed about 20 g) were kept in separate cages. Group I received an intraperitoneal injection of 30 g of Camelyn M per mouse (0.1 ml of Camelyn M solution at 300 g/ml), and group II received 60 g of the drug per mouse (0.1 ml of Camelyn M solution at 600 g/ml). After 3 hours, each group (I and II) was injected with 50 MLD *of S. typhimurium* NCTC 74 bacilli. A control group of 60 mice was injected with the same bacterial strain, and instead of Camelyn M product, 0.1 ml of sterile saline was injected in this group each.

The protective ability of the drug was determined by recording the mortality of mice in different groups up to 100 hours after administration of the drug and bacteria. A χ^2test was used in the statistical analysis of the results obtained. In another experiment, 4 groups of mice were used, with 5 animals per group. Mice in groups 1 and 3 were each given 60 g of Camelyn M product, while mice in groups 2 and 4 were each given 0.1 ml of sterile saline.

After 3 hours, mice from all groups were each given 50 MLD *of S. typhimurium* NCTC 74 bacilli. After [another] 2 hours, mice from groups 1 and 2 were killed. Blood from the heart was collected aseptically from the killed mice; livers and spleens were also collected aseptically from these mice, which were homogenized in tissue homogenizers. The CFU values of each organ were determined separately. The same procedures were carried out in groups 3 and 4, 18 hours after administration of the bacteria. Statistical analysis of the *in vivo* data was performed using Student*'s t* test.

The concentration of Camelyn M in the blood of mice was assessed by measuring the diameter of the zones of inhibition using serum-soaked filter paper discs (6 mm diameter, 3 mm thick, Millipore, absorbent volume 0.03 ml) on a surface flooded with bacteria from an 18-hour culture of *S. typhimurium* 74 in broth on peptone agar. Serum drug concentrations were determined by relating these values to a standard calibration curve prepared for known drug concentrations.

Results:

All bacterial strains tested were found to be resistant to multiple antibiotics. In contrast, the Camelyn M product showed strong antimicrobial activity against all bacteria. Camelyn M product in the concentration range of 0.156-3.0 μg/ml inhibited all bacterial strains:

Figure 1. MIC values of Camelyn M product for **specific antibiotic-resistant bacteria** determined by broth dilution method using Mueller Hinton broth.

Vertically - bacterial strains:

A) - *E. faecium* SF11770
B) - *B. antracis Chad18*
C) - *S. aureus* BM3318
D) - *B. antracis JPS854*
E) - *S.typhimurium* NCTC 74
F) - *S. sciuri* CSLA3
G) - *S. haemolyticus* VPS617
H) - *B. cereus* AND934

Horizontal - MIC values of Camelyn M product.

1) - 0.002
2) - 0.004
3) - 0.009
4) - 0.019
5) - 0.039
6) - 0.078
7) - 0.156
8) - 0.310
9) - 0.62
10) - 1.25
11) - 2.50

Figure 2. MIC values of Camelyn M product for *Bacilus anthracis* resistant to specific antibiotics - A - after 4-hour inoculation and B - after 4-hour inoculation**.**

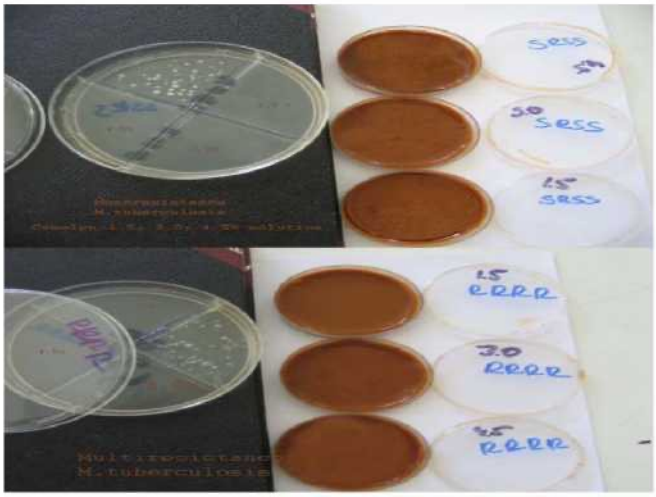

Fig. 3. MIC values of Camelyn M product against individual strains of *M. tuberculosis* - single-drug resistant and multidrug resistant.

***In vitro* antifungal activity.**

Table 1 shows the spectrum of activity of Camelyn M and other reference products against various fungal strains. The Camelyn M product showed strong activities against *C. albicans, C. glabrata, C. tropicalis, C. guilliermondii* and *C. neoformans*, with MIC values ranging from 0.012 µg/ml for all of the aforementioned cases. Table 2 shows the MIC values of Camelyn M and other reference agents for the clinical yeast isolate *C. albicans.* Table 2 shows the results for *C. albicans,* separately for FLC-sensitive (FLC-S) (FLC MIC ≤8 µg/ml) and FLC-sensitive in a dose-dependent manner (FLC-S-DD) and FLC-resistant (FLC-R) strains (FLC MIC≥ 16 µg/ml), according to the guidelines of NCCLS document M27-A2 (14). The Camelyn M product showed potent activity against *C. albicans* (FLC-S), with an MIC value at which inhibition of 90% of the isolate was achieved (MIC_{90s}) of 0.012 µg/ml. The Camelyn M product also showed strong activity against strains of *C. albicans* FLC-S-DD and FLC-R (MIC range, 0.012 µg/ml).

TABLE 1. Antifungal spectrum of Camelyn M product

Organism and strain	MIC (µg/ml)			
	Camelyn M	FLC	ITC	AMB
Candida albicans ATCC 24433	0.012	0.25	0.016	0.12
Candida glabrata ATCC 90030	0.012	4.00	0.12	0.12
Candida tropicalis ATCC 750	0.012	2.00	0.06	0.25
Candida parapsilosis ATCC 22019	0.012	2.00	0.03	0.5
Candida krusei TIMM3378	0.012	32.00	0.06	0.25
Candida guilliermondii ATCC 9390	0.012	2.00	0.03	0.06

TABLE 2. *In vitro* antifungal activity of Camelyn M against a clinical isolate of *Candida albicans.*

Organism (clinical isolates) and agent.	MIC (µg/ml)	
	50%	90%
Candida albicans		
"Camelyn M"	0.012	0.012
FLC	0.25-8	1.00
ITC	0.016	0.03
AMB	0.12	0.12

Camelyn M product belongs to natural antibacterial and antifungal agents with a complex mechanism of action. It is very interesting that the Camelyn M product contains very biologically active phenolic compounds. These compounds, which are found in the leaves and rhizomes of many plants, provide resistance of these plants to various pathogens. Natural phenolic compounds are characterized by strong detergent properties and, when present in small organisms, cause destruction of the cell membrane. Based on the above data, it can be assumed that the biological activity of the Camelyn product involves the above mechanisms, which is the subject of in-depth research.

As can be seen from Tables 1 and 2, the Camelyn M product shows sine *in vitro* activity against all tested strains at the same concentration - MIC (µg/ml)= 0.012. This is lower than the concentrations of all the antibiotics we tested.

In conclusion, the results of the current study suggest that Camelyn M is a promising product, particularly for the treatment of disseminated or bacterial infections and mucosal infections caused by *C. albicans*, including FLC-resistant strains.

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