Antifungal efficacy of natural antiseptic products against *Candida auris*

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ABSTRACT

Candida auris (*C. auris*) is an emerging fungal pathogen responsible for healthcare associated infections and outbreaks with high mortality around the world. It readily colonizes the skin, nares, respiratory and urinary tract of hospitalized patients, and such colonization may lead to invasive *Candida* infection in susceptible patients. However, there is currently a lack of decolonization protocol for *C. auris*. The aim of this study is to evaluate the susceptibility of *C. auris* to commonly used synthetic and natural antiseptic products. Synthetic antiseptics including chlorhexidine, povidone-iodine, and nystatin were shown to be fungicidal against *C. auris*. Among the natural antiseptics tested, tea tree oil and manuka oil were fungicidal at concentrations less than 1.25% and 0.63% (v/v), respectively. Manuka honey inhibited *C. auris* at 25% (v/v) concentrations. Among the commercial products tested, manuka body wash and mouthwash were fungicidal against *C. auris* at concentrations less than 0.10% (w/v) and 6.25% (v/v), respectively, while tea tree body wash and Medihoney™ wound gel demonstrated fungistatic properties. In conclusion, this study demonstrated good in vitro antifungal efficacy of tea tree oil, manuka oil, manuka honey, and commercially available antiseptic products containing these active ingredients. Future studies are warranted to evaluate the effectiveness of these antiseptic products in clinical settings.

INTRODUCTION

The fungal pathogen *C. auris* was first described in 2009 after isolation from the ear of a patient in Japan [1]. It has since been reported in more than 40 countries across six continents, causing a wide range of healthcare-associated outbreaks and infections [2], with the mortality rate varying from 30 to 60%, due to the high virulence and resistance to several antifungals [3]. The recent inaugural World Health Organization fungal priority pathogen list highlights the urgent threat of *C. auris* to global public health [4]. With its biofilm-forming ability and tolerance to high temperature and salt concentrations, *C. auris* can persist and survive in the human skin and environmental surfaces for several months following exposures [5-7]. This provides additional opportunities for transmission and colonization within the healthcare settings. Furthermore, the minimum time to acquire *C. auris* from a patient or their immediate environment is 4 hours or less, reinforcing the importance of vigilant infection control measures [8]. This fungal superbug can also resist certain disinfectants and is well adapted to healthcare environments [9].

In 2019, a cluster of *C. auris* colonization affecting 15 patients was reported in Princess Margaret Hospital (PMH), Kowloon West (KW) cluster, Hong Kong. Whole genome sequencing identified the strains as Clade 1/South Asia, associated with ERG11 mutation conferring azole resistance [10]. The clade is also known to cause invasive infections and outbreaks frequently [6]. Four regional hospitals (a total of 3,878 beds) serve an estimated population of 1.4 million in the KW cluster. In spite of enhanced surveillance and infection control measures, *C. auris* cases and transmission have continued to increase due to pandemic-related strain on the healthcare system. As of April 2023, there were 364 cases of *C. auris* reported in KW Cluster, Hong Kong.

C. auris readily colonizes the axillae, groin, nares, respiratory, and urinary tract of hospitalized patients, and less frequently the intestinal tract than other *Candida* species [11].

Colonization predisposes to invasive infection, with mechanical ventilation and placement of invasive devices identified as major risk factors [6]. A descriptive analysis of *C. auris* outbreaks in New York State reported that 4% of *C. auris* colonized patients developed bloodstream infection, with a rate of 0.3 per 1,000 patient-days [12]. These studies provided evidence that an important strategy for preventing severe candidiasis is the elimination of *C. auris* carriage to preclude subsequent risk of endogenous infection. Nevertheless, recommendations on decolonization protocol for *C. auris* are currently lacking. Successful decolonization by a five-day protocol with twice-daily chlorhexidine body wash, mouthwash containing 0.2% chlorhexidine, and oral nystatin has been reported in a healthcare worker having positive nasal swabs [8]. Both chlorhexidine, povidone-iodine, and nystatin have been used as cutaneous antiseptics prior to surgical procedures, wound irrigation, and the insertion of intravascular devices [13-16]. In search of an effective decolonization protocol for *C. auris*, different topical antiseptics, such as chlorhexidine, povidone-iodine, isopropyl alcohol, and nystatin have been studied, with their yeasticidal activities against *C. auris* demonstrated in vitro [17-19]. Chlorhexidine is one of the most common disinfectants used in healthcare settings. Despite laboratory evidence showing excellent in vitro activity of chlorhexidine against *C. auris*, clinical experience from several outbreaks suggested that colonization persisted even with daily chlorhexidine washes [8, 9]. A murine model study demonstrated that *C. auris* could enter the dermis without causing overt histopathologic signs of inflammation, resulting in deeper skin layer residence [20]. One factor contributing to *C. auris* persistent colonization may be the limited permeation into deeper skin layers by chlorhexidine, which was illustrated by a study based on a model of ex vivo human skin [21]. Poor effectiveness of chlorhexidine in decolonization, as well as subsequent recolonization of *C. auris* in patient who had repeated interval negative swabs, is anticipated.

The development of novel and effective decolonization regimen against *C. auris* using

alternative antiseptic products has become crucial. Natural substances with antimicrobial properties are potential good candidates as they would not mount additional drug-selecting pressure and they are well tolerated as skin washes in general. *Fernades et al.* have shown that tea tree (*Melalenca alternifolia*) oil (TTO) had good in vitro antifungal activity against *C. auris* in both planktonic cells and biofilms [22]. *Johnson et al.* have also shown that the active components of TTO could augment the activity of chlorhexidine for *C. auris* decolonization in the porcine skin model [23]. Another potential effective natural antiseptic is manuka (*Leptospermum scoparium*), which is also known as the "New Zealand tea tree". It has been a part of the traditional Māori medicine, including topical treatment for wounds, cuts, and sores, with fungicidal properties towards *C. albicans* and *C. glabrata* [24]. Notably, manuka honey was found to be effective in inhibiting various *Candida* species, including *C. auris* [25, 26]. There are multiple antimicrobial constituents of the natural extracts, namely monoterpenes and sesquiterpenes (TTO and manuka oil), triketones (manuka oil), methylglyoxal, polyphenolic compounds and bee defensin-1 (manuka honey) [27-29]. Therefore, unlike other synthetic antimicrobials, there have been no reports of clinical isolates with acquired resistance to the natural antiseptics [30, 31]. Clinical trials have demonstrated the efficacy of manuka honey and TTO in the treatment of oral candidiasis, vulvovaginal candidiasis and successful decolonization for methicillin-resistant Staphylococcus aureus (MRSA) carriers [32-37], making them attractive alternatives against *C. auris*. These natural components are also widely used as active ingredients for their antiseptic effects in commercial products such as body wash, mouthwash and wound gel. To pave the way for evaluating the clinical application of the natural antiseptics against *C. auris*, this study aimed to determine the in-vitro antifungal susceptibility of *C. auris* to the natural antiseptics and their commercial products; and to compare the antifungal susceptibility with that of synthetic antiseptics products.

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MATERIALS AND METHODS

Characterization of *Candida auris* isolates: A total of 40 *C. auris* isolates belonging to clade I/South Asia (unpublished data) were obtained in the KW Cluster of Hong Kong from 2019 to 2022. All tested isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF Biotyper, Bruker Daltonics; Database version: IVD-9468 MSP) [38]. Reference strains *C. auris* JCM 15448, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 22019 were also included. The study protocol was approved by the Research Ethics Committee of the Kowloon West Cluster, Hospital Authority, Hong Kong (KW/EX-21-098(160-14)).

Antiseptic products and preparations: Our experiment studied three groups of topical antiseptic products. The first group included synthetic antiseptics products commonly used in healthcare settings: chlorhexidine gluconate (Microshield® 4%, Australia), povidone-iodine (Betadine® 10%, Turkey) and nystatin (100,000 units/mL, Pharmascience, Canada). The second group comprised natural antiseptics: TTO (15%, The Body Shop, UK; 100% Kiwi Manuka®, New Zealand), manuka oil (100%, Manuka Lab®, New Zealand; 100%, Melora®, New Zealand), manuka honey (UMF 15+ and 20+, Comvita®, New Zealand; UMF 15+ and 20+, Melora®, New Zealand), manuka hydrosol (Bioactives®, New Zealand). The third group consisted of commercial antiseptic products containing active components from TTO and/or manuka derivatives: manuka body wash (Manuka Lab®, New Zealand), tea tree clearing body wash (The Body Shop®, UK), manuka mouthwash (Manuka Lab®, New Zealand), and Medihoney™ antibacterial wound gel (Comvita®, New Zealand). Dimethyl sulfoxide (DMSO, Sigma-Aldrich, UK) was added to water-insoluble products, including TTO, manuka oil, antibacterial wound gel, and nystatin to enhance solubility in RPMI 1640 medium (Sigma, Paisley, UK).

In vitro antifungal susceptibility of antiseptic products: In vitro antifungal

susceptibility of antiseptic products was performed by broth microdilution method according to CLSI M27-A4 [39]. Briefly, 100 μL of RPMI 1640 medium with different concentrations of the antiseptic products were added to 96-well microtiter plates (Saining Biotechnology, China). *Candida* isolates were suspended in 2 mL sterile physiological saline to obtain 0.5 McFarland standard suspension using the DensiCHEK Plus instrument (BioMerieux, France), corresponding to 1-5 x 10^6 cells per mL, then further diluted with RPMI 1640 medium to obtain a 1:1000 final inoculum containing 1-5 x 10^3 cells per mL. The yeast suspensions (100 μL) were added to the 96-well plate with the product suspensions (100 μL) and incubated aerobically at 35°C for 24 h. The minimum inhibitory concentration (MIC) endpoint of a product was defined as the lowest drug concentration that caused a prominent decrease in growth (50%) in relation to the growth control using a reading mirror. The minimum fungicidal concentration (MFC) endpoint was defined as the lowest drug concentration resulting in the death of 99.9% or more of the initial inoculum. To determine MFC values, 100 μL of broth was taken from the wells without microbial growth and inoculated onto Sabouraud dextrose agar (BD DIFCO™, USA). Following incubation at 35°C for 48 h, the colony-forming unit (CFU) was counted to assess viability. Each isolate was tested in duplicate separately and the assay was repeated if the MIC or MFC differed by more than one dilution. Antifungal drug susceptibility of *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 was performed in each run of broth microdilution as controls [40].

For antiseptic products demonstrating fungistatic effects without fungicidal (MFC) endpoints, viable cell count was assessed and expressed in log growth reduction in CFU/mL after 24 h of incubation at 35°C, and the test was repeated in triplicate. Mann-Whitney U test was used to analyze the significance of difference in CFU count compared to the growth control, and one-way ANOVA, Kruskal-Wallis, or Mann-Whitney U tests were used to compare the log growth reduction of the antiseptic products at different concentrations.

Pairwise comparisons were performed using Bonferroni or Dunn's test when suitable. P value <0.05 was considered statistically significant. All statistical analyses were performed using STATA18.0 (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC.).

In vitro antifungal susceptibility testing of antifungal drugs: In vitro antifungal susceptibility was determined using Sensititre™ YeastONE™ YO10 AST Plate (ThermoFisher Scientific, UK). The antifungal drugs included anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole and amphotericin B. The test was performed according to the procedures described by the manufacturer [41]. Centres for Disease Control and Prevention (CDC)'s tentative breakpoints for *C. auris* were used as a reference [42].

RESULTS

Table 1 summarizes the antifungal activities of topical antiseptic products against *C. auris*. For the synthetic antiseptic products, chlorhexidine, povidone-iodine and nystatin were fungicidal against *C. auris* at concentrations less than 21.99 mg/L (0.004%), 312.50 mg/L (0.313%), and 9.77 mg/L (0.049%), respectively. The MICs and MFCs of chlorhexidine against *C. auris* strains, including the JCM 15448, were relatively higher when compared with that against *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

For the natural antiseptics, manuka honey demonstrated fungistatic properties against clinical *C. auris* isolates at 25% (v/v), while the fungistatic inhibition against *C. auris* JCM 15448 strain was observed at a much lower concentration at 0.20% (v/v). Pure TTO and manuka oil were fungicidal against *C. auris* at concentrations less than 1.25% and 0.63% (v/v), respectively. Most of the commercial antiseptic products containing active ingredients of manuka honey, manuka oil and/or TTO were shown to be active in vitro against *C. auris* and other *Candida* species. Manuka Lab® manuka body wash and mouthwash were fungicidal against *C. auris* at concentrations less than 0.10% (w/v) and 6.25% (v/v), respectively. Manuka body wash, however, exhibited weaker antifungal activity against *C. parapsilosis* (MFC 3.13% (w/v)). The Body Shop® tea tree body wash and Medihoney™ wound gel inhibited clinical *C. auris* isolates at concentrations less than 0.39% (w/v) and 6.25% (w/v), respectively. Interestingly, the MICs of natural antiseptics against *C. auris* JCM 15448 were 4- to 128-fold lower than that against the clinical counterparts; tea tree body wash and wound gel even became fungicidal against *C. auris* JCM 15488 at concentrations of 25% (w/v) and 12.5% (w/v), respectively. A similar increase in susceptibility of *C. krusei* was also observed for tea tree body wash and Medihoney™ wound gel. Manuka hydrosol was not effective against all the tested *Candida* species.

Manuka honey, Medihoney™ wound gel and tea tree body wash demonstrated

fungistatic properties against clinical *C. auris* isolates in vitro. At a concentration of 37.5%, the natural antiseptic products exhibited an average 4 log reduction in CFU after 24 h of incubation (Figure 1), which is statistically significant in comparison with the growth control ($p < 0.01$). The inhibitory effects of manuka honey and tea tree body wash against *C. auris* were concentration-dependent ($p < 0.05$). In contrast, concentrations of 25% and 37.5% (w/v) Medihoney™ wound gel resulted in a similar reduction in CFU (6 log).

The antifungal susceptibility of common systemic antifungals against *C. auris* was shown in table 2. All tested isolates (N=40) were resistant to fluconazole and 90% (N=36) were also resistant to Amphotericin B. Susceptibility to other azoles and echinocandins was variable.

DISCUSSION

The emergence of multidrug-resistant *C. auris* to conventional antifungal drugs and its high propensity to cause nosocomial transmission make it urgent to develop novel and effective decolonization strategy. Herein, we demonstrated excellent in vitro antifungal activity of natural antiseptics, including manuka honey, manuka oil and TTO, and their commercial hygienic products, against *C. auris*. This suggests the current decolonization strategy could be much improved by combining these well-tolerated, natural antiseptic products.

In the present study, the local *C. auris* isolates were highly resistant to both fluconazole (MIC_{50} of 256 μg/mL) and amphotericin B (MIC_{50} of 2 μg/mL). Genomic and proteomic analyses revealed mutation of the fluconazole target enzyme (cytochrome 450 lanosterol 14α-demethylase) [10] and the role of extracellular vesicles in the development of amphotericin B resistance [43].

Our results confirm the high susceptibility of *C. auris* to chlorhexidine (MFC 0.004%), povidone iodine (MFC 0.312%) and nystatin (MFC 9.77 μg/mL) at concentrations used for clinical indications [44, 45]. Likewise, *C. auris* remained highly susceptible to TTO and manuka oil, with MFC less than 1.25% and 0.63% (v/v), respectively; the fungicidal effects of TTO and manuka oil were comparable to synthetic antiseptics, including chlorhexidine, povidone-iodine and nystatin. Our findings are consistent with a previous report of high antifungal activity of TTO against *C. auris* isolates (MFC 1.56% (w/v)) [46]. Essential oil of tea tree has been shown to cause damage to the fungal cell wall and cytoplasmic membrane thereby causing cellular dysfunctions and death though the exact mechanism is unknown [47, 48]. Interestingly, The Body Shop® formulation contains a diluted content of 15% TTO but a lower MIC_{90} of 0.004% (v/v) was observed compared to 100% TTO. Other important ingredients listed by Body Shop® TTO are denatured alcohol, *Calophyllum inophyllum* seed

oil, *Leptospermum petersonii* oil, and tocopherol. This enhanced inhibition of *C. auris* may be attributed to the synergistic or additive interactions of these supplementary components as various studies have demonstrated the antimicrobial properties of *Calophyllum inophyllum* oil and *Leptospermum petersonii* oil [49, 50]. *Moore et al.* also showed the addition of isopropyl alcohol to disinfectant formulation clearly enhanced the killing of *C. auris* [17]. On the other hand, the antifungal properties of manuka oil were less frequently evaluated for *C. auris* specifically. The significant inhibitory effects of manuka oil on *C. auris* AR0381 (clade II) and AR0385 (clade IV), with MIC values less than 1.0% [51], agree well with our findings. More importantly, the low fungicidal concentrations of TTO and manuka oil against *C. auris* are considered safe and tolerable for dermal use. An in vitro skin model proved manuka oil is non-irritant, with cell viability unaffected after treatment with an undiluted sample [52]. Another clinical trial also demonstrated that 5% TTO shampoo was well tolerated in the treatment for dandruff [53]. The additional property of enhanced skin permeation of essential oils [54] further supports the role of essential oils in eliminating *C. auris* colonization.

Regarding manuka honey, *C. auris* were inhibited at a concentration of 25%. The growth inhibition was presented in a dose-dependent manner. This is in line with several studies showing fungistatic effect of manuka honey against *Candida* species. [26, 46]. Manuka honey belongs to the "non-peroxide-based" honey group, and its main antimicrobial action is related to the antimicrobial molecule methylglyoxal (MGO) [55], which is converted non-enzymatically from the dihydroxyacetone present in the nectar of the flowers of the manuka tree (*Leptospermum scoparium*). The characteristically high level of MGO in manuka honey makes it a unique marker for authentication. Research suggested that the antimicrobial potency of manuka honey is related to the Unique Manuka Factor (UMF) rating, which is correlated with the MGO and total phenol components [56]. In this study, manuka honey with greater UMF resulted in greater reduction of *C. auris* CFU. Increasing the concentration of

honey from 25% to 50% led to an average 3-log reduction in the CFU of *C. auris* ($p < 0.05$). Surprisingly, the *C. auris* JCM 15448 (clade II) had a much lower MIC (0.2% v/v) towards manuka honey. Similar phenomenon was also observed for other natural antiseptic products. Nevertheless, previous study did not suggest difference in antifungal activity of medical grade honey formulation among *C. auris* clades [25]. Studies including larger numbers of *C. auris* of different clades should determine whether susceptibility to natural antiseptics is clade specific.

As expected, commercial products having the active ingredients of natural antiseptics showed promising antifungal effect in vitro against *C. auris*. Manuka body wash and mouthwash from Manuka Lab® were fungicidal against *C. auris* at concentrations of 0.1% (w/v) and 6.25% (v/v), respectively. Product labels of manuka body wash and mouthwash quotes the content of 525+ MGO (mg/kg), which is equivalent to UMF 15+ manuka honey. Besides, the aforementioned products contain TTO and manuka oil with antifungal effects demonstrated in our study. Alternatively, Medihoney™ antibacterial wound gel (80% medical-grade manuka honey) and The Body Shop® tea tree body wash, also caused a 6-log reduction in *C. auris* CFU. Together with the anti-inflammatory and anti-oxidant properties of manuka honey [29], the commercial hygienic products serve as important options in the development of an efficacious and tolerable decolonization regimen.

There are some limitations in this study. First, extraction and evaluation of the bioactive chemical markers in the natural antiseptic products were not performed. The specific quantities of the active ingredients were often not disclosed in the product labels. Besides, all our *C. auris* isolates belonged to clade I and the results of this study may not be applicable to isolates of other clades. We had included *C. auris* JCM 15448, a clade II isolate, in our testing and found significantly lower MIC and MFC values towards the natural antiseptics. Moreover, the effect of antifungal drug resistance on the antiseptic products could not be

studied due to the low variability of the resistance pattern of our *C. auris* isolates. Nonetheless, the antiseptic products had an overall high in vitro antifungal efficacy, which supported their application as topical or decolonization therapies for *C. auris.*

CONCLUSION

C. auris are highly susceptible to natural antiseptics including TTO, manuka oil and manuka honey, and their commercial hygienic products in the forms of body wash, mouthwash, and wound gel. The promising in vitro results provide insight into the development of novel and effective *C. auris* decolonization regimen. Future studies should demonstrate whether these natural antiseptics are effective in the clinical settings.

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CONFLICT OF INTEREST

We declare no conflict of interest.

DATA AVAILABILITY

All data are incorporated into the article and its online supplementary material.

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499 **LEGENDS TO TABLE AND FIGURE**

500 **Table 1:** In vitro susceptibility of *Candida* isolates to synthetic antiseptics products, natural antiseptics and their commercial products

	Clinical *C. auris* isolates (N=40)				*C. auris* JCM 15448		*C. krusei* ATCC 6258		*C. parapsilosis* ATCC 22019	
	MIC_{50}	MIC_{90}	MIC range	MFC range	MIC	MFC	MIC	MFC	MIC	MFC
Chlorhexidine (%)	0.001	0.001	0.0005-0.001	0.002-0.004	0.001	0.002	0.0002	0.0005	0.00006	0.0002
Povidone-iodine (%)	0.078	0.078	0.039-0.078	0.078-0.313	0.039	0.156	0.039	0.313	0.078	0.625
Nystatin (µg/mL)	2.44	2.44	1.22-2.44	2.44-9.77	1.22	1.22	1.22	2.44	0.61	2.44
Manuka honey 15+ (Comvita) (v/v) (%)	25	25	6.25-25	>50	0.20	>50	25	>50	25	>50
Manuka honey 20+ (Comvita) (v/v) (%)	25	25	3.13-25	>50	0.20	>50	12.5	>50	25	>50
Manuka honey 15+ (Kiwi Manuka) (v/v) (%)	25	25	3.13-25	>50	0.20	>50	12.5	>50	25	>50
Manuka honey 20+ (Kiwi Manuka) (v/v) (%)	25	25	6.25-25	>50	0.20	>50	12.5	>50	25	>50
Tea tree oil 15% (Body Shop) (v/v) (%)	0.004	0.004	0.004	2.08-4.17	0.004	1.04	0.002	2.08	0.004	4.17
Tea tree oil 100% (Kiwi Manuka) (v/v) (%)	0.31	0.31	0.31	0.63-1.25	0.31	0.63	0.63	1.25	0.63	1.25
Manuka oil 100% (Manuka Lab) (v/v) (%)	0.31	0.31	0.16-0.31	0.63	0.08	0.63	0.31	1.25	0.31	1.25
Manuka oil 100% (Melora) (v/v) (%)	0.31	0.31	0.31	0.63	0.31	0.31	0.31	1.25	0.31	1.25
Manuka hydrosol (v/v) (%)	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Manuka body wash (w/v) (%)	0.01	0.01	0.006-0.01	0.05-0.10	0.006	0.02	0.006	0.05	0.006	3.13
Tea tree body wash (w/v) (%)	0.012	0.012	0.006-0.39	≥50	0.003	25	0.002	25	0.003	50
MEDIHONEY wound gel (w/v) (%)	3.13	3.13	1.56-6.25	≥25	0.20	12.5	0.39	25	0.20	>37.5
Manuka mouthwash (v/v) (%)	0.39	0.39	0.20-0.39	3.13-6.25	0.39	6.25	0.10	6.25	0.20	6.25

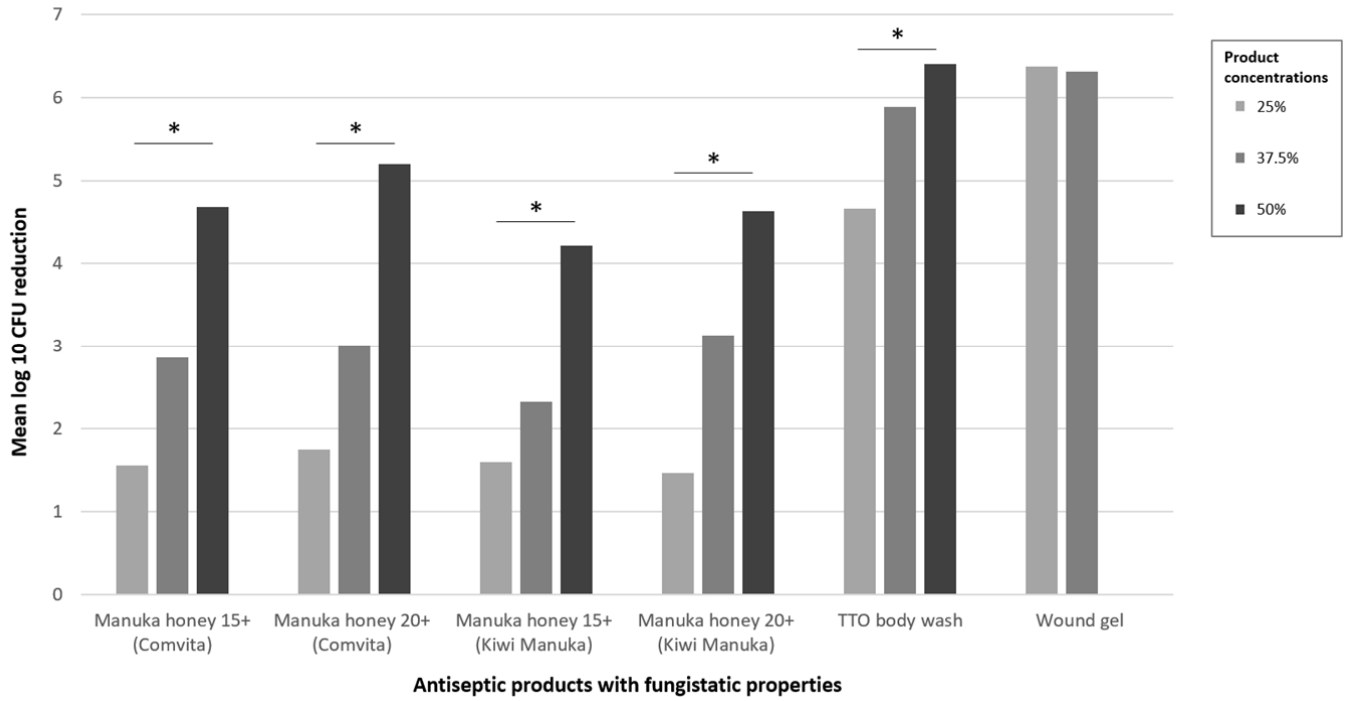

Figure 1: The fungistatic effect of natural antiseptic products on *Candida auris*. Three *C. auris* isolates were incubated with 4 types of manuka honey, tea tree body wash – 25%, 37.5% and 50%, and antibacterial wound gel – 25% and 37.5% (50% wound gel is not available for testing due to its insolubility at high concentrations). Log reductions in CFU were shown. Significant differences ($p < 0.05$) are indicated with an asterisk.

507 **Table 2:** Antifungal drug susceptibility of *C. auris* isolates

	Clinical *C. auris* isolates (N=40)			*C. auris* JCM 15448
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	MIC (mg/L)
Amphotericin B	2	4	1-4	1
Fluconazole	256	>256	64 - >256	8
Anidulafungin	0.25	0.25	0.12 - >8	0.12
Micafungin	0.25	0.25	0.06 - 1	0.06
Caspofungin	0.25	0.5	0.06 - >8	0.03
Itraconazole	0.12	0.25	0.06 - >16	0.06
Voriconazole	0.5	1	0.12 - >8	0.06
Posaconazole	0.03	0.06	0.015 - >8	0.03
5-flucytosine	0.12	0.12	<0.06 - >64	0.25

508

509 CDC's tentative nonsusceptible breakpoints: ≥2 mg/L for amphotericin B, ≥32 mg/L for fluconazole, ≥4 mg/L for echinocandins anidulafungin

510 and micafungin and ≥2 mg/L for caspofungin. For the triazoles itraconazole, voriconazole and posaconazole, and 5-flucytosine, no breakpoints

511 are available.

512

513 **SUPPLEMENTARY MATERIAL 1:** Natural and commercial tested products with ingredients as per label disclosure

Natural and commercial tested products	Major antiseptic ingredient(s)	Other ingredient(s)
Manuka oil (Melora®)	Manuka (Leptospermum scoparium) branch/leaf oil	
Manuka oil (Manuka Lab®)	Manuka (Leptospermum scoparium) branch/leaf oil	
Manuka hydrosol (Bioactive®)	Manuka (Leptpspermum scoparium) branch/flower/leaf water	
Tea tree oil (Kiwi Manuka®)	Tea tree (Melaleuca alternifolia) leaf oil	
Tea tree oil (The Body Shop®)	Tea tree (Melaleuca alternifolia) leaf oil	Aqua, denatured alcohol, polysorbate 60, limonene, Calophyllum Inophyllum seed oil, Leptospermum Petersonii oil, citral, citronellol, denatonium benzoate and tocopherol
Triple action Manuka body wash (Manuka Lab®)	Manuka honey, manuka (Leptospermum scoparium) branch/leaf oil, tea tree (Melaleuca alternifolia) leaf oil	Aqua, ammonium lauryl sulfate, cocamidopropyl betaine, lauryl glucoside, citric acid, capryl glucoside, glycerin, sodium benzoate, potassium sorbate, sodium phylate, Hordeum Vulgare leaf extract, Triticum Aestivurn leaf extract
Triple action Manuka mouthwash (Manuka Lab®)	Manuka honey, manuka (Leptospermum scoparium) branch/leaf oil, tea tree (Melaleuca alternifolia) leaf oil	Aqua, sorbitol, polysorbate 20, glycerin, Mentha Piperita oil, Barbadensis Leal juice powder, Triticum Aestivum leaf extract, Hordeum Vulgare leaf extract, menthol, xylitol, caramel, phenoxyethanol, ethylexylglycerin, Capsicum Annuum fruit extract, limonene
Tea Tree Skin Clearing body wash (The Body Shop®)	Tea tree (Melaleuca alternifolia) leaf oil	Aqua, sodium laureth sulfate, sodium chloride, peg-40 hydrogenated castor oil, polysorbate 20, cocamidopropyl betaine, peg-120 methyl glucose dioleate, phenoxyethanol, glycerin, sodium benzoate, Calophyllum Inophyllum seed oil, citric acid, butyl methoxydibenzoylmethane, disodium EDTA, limonene, sodium hydroxide, Leptospermum Petersonii oil, tocopherol, caramel
Medihoney™ antibacterial wound gel (Comvita®)	Manuka honey	Myristyl myristate, capryl glucoside

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515 **SUPPLEMENTARY MATERIAL 2:** Pairwise comparisons of log CFU reduction of *C. auris* isolates by differing concentrations of fungistatic

516 antiseptic products.

Reagents	p-value
Manuka honey (Comvita®) 15+	
25% vs. 37.5%	<0.001
37.5% vs. 50%	<0.001
25% vs. 50%	<0.001
Manuka honey (Comvita®) 20+	
25% vs. 37.5%	0.009
37.5% vs. 50%	0.007
25% vs. 50%	<0.001
Manuka honey (Kiwi Manuka®) 15+	
25% vs. 37.5%	0.009
37.5% vs. 50%	0.007
25% vs. 50%	<0.001
Manuka honey (Kiwi Manuka®) 20+	
25% vs. 37.5%	<0.001
37.5% vs. 50%	<0.001
25% vs. 50%	<0.001
Tea tree body wash (The Body Shop®)	
25% vs. 37.5%	0.003
37.5% vs. 50%	0.061
25% vs. 50%	<0.001

517