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Antifungal Activity Exerted by Greek Honeys and Bacteria Isolated from Them
Yunan Ballarının ve Bunlardan İzole Edilen Bakterilerin Antifungal Aktivitesi

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Abstract

A plethora of studies provide evidence regarding honey's biological properties such as antibacterial, antioxidant, and anti-inflammatory activity. However, antifungal activity exerted by honey is rather under investigated. Due to widespread antimicrobial resistance, the emergence of novel antifungal agents, as well as the identification of alternative therapies, is crucial. This study aimed to investigate the antifungal activity exerted by heather and chestnut honeys, harvested across Greece, as well as the antifungal activity of bacteria isolated from them, against *Penicillium commune*, *Penicillium expansum*, *Aspergillus niger*, *Candida albicans M10/20* and *Candida albicans M 351/19*. Antifungal activity of tested honeys was evaluated by Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) whereas antifungal activity of bacterial isolates by antagonism assay. Fungistatic activity against all tested fungi and fungicidal activity against *C. albicans* strains was exerted by most Greek honeys. Exerted antifungal activity was comparable to Manuka honey. Furthermore, most of the identified bacterial isolates inhibited the growth of fungal strains, in antagonism assays. This study for the first time demonstrated the significant antifungal activity exerted by heather and chestnut honey produced in Greece, as well as the important role of their microbiome in observed antifungal activity. Nevertheless, our results warrant further research in order to develop novel antifungal agents and alternative therapies.

Keywords: Greek Heather Honey, Greek Chestnut Honey, Antifungal activity, Bacterial isolates, *Candida albicans*, food spoilage fungi.

Özet

Çok sayıda çalışma, balın antibakteriyel, antioksidan ve anti-inflamatuar aktivite gibi biyolojik özellikleriyle ilgili kanıtlar sunmaktadır. Ancak, balın antifungal aktivitesi oldukça az araştırılmıştır. Yaygın antimikrobiyal direnç nedeniyle, yeni antifungal ajanların ortaya çıkması ve alternatif tedavilerin tanımlanması hayati önem taşımaktadır. Bu çalışma, Yunanistan genelinde hasat edilen funda ve kestane ballarının ve bunlardan izole edilen bakterilerin *Penicillium commune, Penicillium expansum, Aspergillus niger, Candida albicans M10/20 ve Candida albicans M 351/19'a* karşı uyguladığı antifungal aktiviteyi araştırmayı amaçlamaktadır. Test edilen balların antifungal aktivitesi Minimum İnhibitör Konsantrasyon (MİK) ve Minimum Fungisidal Konsantrasyonun (MFC) Belirlenmesi ile değerlendirilirken, bakteri izolatlarının antifungal aktivitesi antagonizm testi ile değerlendirilmiştir. Test edilen tüm mantarlara karşı fungistatik aktivite ve *C. albicans* suşlarına karşı fungisidal aktivite çoğu Yunan balı tarafından uygulandı. Uygulanan antifungal aktivite Manuka balına benzerdi. Dahası, tanımlanan bakteri izolatlarının çoğu, antagonizm analizlerinde mantar suşlarının büyümesini engelledi. Bu çalışma, Yunanistan'da üretilen funda ve kestane balının uyguladığı önemli antifungal aktiviteyi ve gözlemlenen antifungal aktivitede mikrobiyomlarının önemli rolünü ilk kez gösterdi. Yine de, sonuçlarımız yeni antifungal ajanlar ve alternatif tedaviler geliştirmek için daha fazla araştırmayı gerektiriyor.

Anahtar Kelimeler: Yunan Funda Balı, Yunan Kestane Balı, Antifungal aktivite, Bakteriyel izolatlar, *Candida albicans*, gıda bozulma mantarları

Abbreviations: MIC; Minimum Inhibitory Concentration, MFC; Minimum Fungicidal Concentration

1. INTRODUCTION

Honey is a natural product highly appreciated for its exceptional nutritional value and bioactivity. It has been used as a traditional remedy due to antimicrobial activity and wound-healing properties since ancient times. Greek honey types exert high antibacterial and antioxidant activity, verified by several studies (Anthimidou & Mossialos, 2013; Stagos et al., 2018; Tsavea et al., 2022; Tsavea & Mossialos, 2019). Physicochemical characteristics including high sugar content, low pH, hydrogen peroxide content, as well as antimicrobial peptides present in honey, modulate the antibacterial, antioxidant, and anti-inflammatory activity (Ranneh et al., 2021; Tsadila et al., 2021). However, the efficacy of honey as an antimicrobial agent has been reported to be highly variable depending on the botanical and geographic origin (Almasaudi, 2021; Ramos et al., 2018; Schiassi et al., 2021).

Chestnut honey, produced from a mixture of *Castanea sativa* nectar and honeydew by *Myzocallis castanicola,* has been shown to inhibit the growth of a diverse range of pathogens, including several *Bacillus* strains (Kačániová et al., 2022) and fungi like *Candida parapsilosis, Candida tropicalis, Cladosporium cladosporioides,* and *Rhodotorula mucilaginosa,* as

described by Kunčič et al. (2012). Likewise, heather honey, derived from *Erica manipuliflora* nectar exerts promising antimicrobial activity. Feás & Estevinho (2011) reported that monofloral heather harvested in Portugal exerted in a concentration-dependent manner antifungal efficacy against *Candida albicans*, *Candida krusei*, and *Cryptococcus neoformans* that was attributed to phytochemicals like polyphenols and flavonoids present in these honeys.

Honey from the Manuka tree (*Leptospermum scoparium*), native to New Zealand and Australia, demonstrates antimicrobial properties, which are not dependent on hydrogen peroxide but on the presence of methylglyoxal, a product of dihydroxyacetone, which affects diverse bacterial proteins and structures including fibria and flagella thus causing bacterial dysfunction (Adams et al., 2009; Rabie et al., 2016). Manuka honey has been extensively studied to date, because of its unique origin and proven ability to inhibit more than 60 bacterial species, including Gram-positive and Gram-negative, aerobic and anaerobic bacteria (Mandal & Mandal, 2011).

Although raw honey exerts antimicrobial activity is not sterile. It contains a unique microbiome consisting of microorganisms coming from plant pollen and nectar, bee digestive tract, and hive milieu (Olaitan et al., 2007). Microorganisms present in honey should adapt under conditions of high osmolarity, low moisture content, low acidity, and endogenous antimicrobial agents. Therefore, the main types of microorganisms surviving in honey are sporulating bacteria and yeasts (Xiong et al., 2023), with *Bacillus* species being the most prominent (Pomastowski et al., 2019; Tsadila et al., 2021). Within the harsh microenvironment of honey, microorganisms compete with each other to access limited resources. Strong competition among diverse microbial species, leads to synthesis and secretion of a multitude of secondary metabolites disrupting key cellular structures and functions of antagonistic microorganisms (Brudzynski, 2021).

Although the antibacterial properties of Greek honey types have been extensively reported (Anthimidou & Mossialos, 2013; Stagos et al., 2018; Tsavea et al., 2022; Tsavea & Mossialos, 2019), the antifungal activity exerted by them is under investigated. Screening of honey exerting antifungal activity and their mode of action is essential for the identification of novel antifungal agents that might combat the emerging antifungal resistance (Lee et al., 2023; Vitiello et al., 2023).

Penicillium and *Aspergillus* species are commonly referred to as “food spoilage fungi” (Snyder & Worobo, 2018). *Penicillium expansum* and *Penicillium commune*, are commonly

grown on fruits and dairy products respectively (Jurado & Vicente, 2020; Tannous et al., 2020). These fungi are highly adaptable in a wide range of conditions, with an optimum growth temperature at 25°C and elevated humidity levels (Li et al., 2020; Pitt & Hocking, 2009b). Both species produce toxins potentially harmful to humans (Pitt & Hocking, 2009b; Vidal et al., 2019). *Aspergillus niger*, identified often as the black mold covering rotten fruits, grows at variable temperatures, optimally at 35-37°C (Pitt & Hocking, 2009a). *A. niger* has been associated with otomycosis and might cause invasive pulmonary aspergillosis in immunocompromised patients (Person et al., 2010; Romsdahl et al., 2018).

On the other hand, *Candida albicans* is a fungal species often present in human oral and gastrointestinal microbiome (Dadar et al., 2018). However, it is an opportunistic pathogen, that under particular circumstances, might cause infections due to dysbiosis of the normal microbiota, immune dysfunction, and damage to the mucosal barrier (Talapko et al., 2021).

The aim of this study was to investigate the antifungal activity of Greek heather and Chestnut honey, along with that of their bacterial isolates, towards the fungi *Penicillium expansum, Penicillium commune, Aspergillus niger,* and *Candida albicans*.

2. MATERIALS and METHODS

2.1. Honey Samples

A total of nine (9) heather and seven (7) chestnut honey samples, harvested across Greece, as shown in Figure 1and Table 1, were provided by individual beekeepers and beekeeping associations. Each sample was recorded by a unique reference number and then was stored in a dry and cool place until testing. In the case of crystalization, samples were warmed up in a waterbath at 35-40 °C for up to 10 min and then stirred. In order to compare the antifungal activity of tested honey samples, Manuka honey UMF 24+ (MGO 1122+) (New Zealand Honey Co), was used as a reference.

Figure 1. Geographical origin of honey samples. Orange map pins indicate chestnut honey samples and green map pins indicate heather honey samples.

Table 1. Information regarding the geographical and botanical origin, as well as, the harvest period of the honey samples.

Reference number	Botanical origin	Geographical origin	Harvest (Month/Year)
200	Chestnut	Veria	07/2020
240	Chestnut	Chania	08/2020
271	Chestnut	Florina	07/2021
187	Chestnut	Fokida	02/2020
267	Chestnut	Serres	07/2021
210	Chestnut	Pella	08/2020
212	Chestnut	Mount Athos	07/2020
229	Heather	Antiparos	12/2020
183	Heather	Kalamos	11/2020
244	Heather	Crete	08/2020
243	Heather	Crete	10/2020
218	Heather	Chania	12/2020
269	Heather	Kavala	04/2021
241	Heather	Chania	10/2020
195	Heather	Ios	11/2020
233	Heather	Andros	01/2021

2.2. Fungal Strains

Penicillium expansum (DSM 62841), *Penicillium commune* (DSM 2211), and *Aspergillus niger* (DSM 2466) strains were purchased by DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. *Candida albicans M10/20* and *Candida albicans M351/19,* isolated from the upper and lower respiratory system respectively, were provided by the A' Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki and they were identified by standard methods.

2.3. Bacterial Strains

Bacterial strains were isolated from diverse honey samples and they were identified by 16S rRNA gene sequencing as described before (Tsadila et al., 2021).

Table 2. Bacterial strains used in this experiment

Strain	Bacteria	Genbank Accession Number
CTA2	*Bacillus pumilus*	MW700013
CTA15	*B. pumilus*	MW700019
CTA31	*Bacillus sp.*	MW700025
CTA163	*Bacillus licheniformis*	MW700039
CTB7	*Bacillus safensis*	MW700041
CTB16	*B.safensis*	MW700043
CTB21	*B.pumilus*	MW700044
CTB89	*B.safensis*	MW700053
CTB120	*B.safensis*	MW700057
CTA20	*Bacillus subtilis*	MW700021
CTB11	*Bacillus sp.*	MW700042
CTA28	*Bacillus paramycoides*	MW700024
CTB34	*Bacillus cereus*	MW700048
CTA23	*Pseudomonas fulva*	MW700022
CTA138	*Bacillus sp.*	MW700037

2.4. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is considered the lowest concentration of honey that completely inhibits fungal growth.

Determination of the minimum inhibitory concentration (MIC) of honey samples was performed on sterile 96-well microtiter plates (Kisker Biotech GmbH & Co. KG, Steinfurt, Germany), as previously described with some modifications (Patton et al., 2006). Spore suspension of *P. commune, P. expansum, A. niger,* or *C. albicans* broth culture, of OD_{530}= 0.09-0.13, was further diluted, using RPMI 1640 broth, w/ L- Glutamine (BioSera, France) at a ratio of 50:1. Subsequently, 100µl of the fungal solution and 100 ml of two-fold diluted honey, were

added inside each well, resulting in final honey concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.562% (v/v). Each honey sample concentration was tested in triplicates. RPMI broth was used as a negative control and RPMI broth inoculated with fungal suspension or fungal culture was used as a positive control for fungal growth. Furthermore, Manuka honey was tested in every microtiter plate, along with the honey samples for comparison. The growth of *C. albicans* strains was calculated by measuring the optical density before (t=0) and after 24h (t=24) incubation at 37°C. Optical density (OD) was determined at 530 nm using an ELx808 Microplate reader ELx808 (BioTek Instruments, Inc., Winooski, VT, USA). In order to determine the percentage of growth inhibition of each honey dilution the following formula was implemented (Patton et al., 2006):

$$100\% \text{ Suspension} = 1 - (\text{DODsample} / \text{DODcontrol}) \times 100$$

Regarding *P. commune, P. expansum, and A. niger*, being filamentous fungi, it was not feasible to measure their growth by optical density. Therefore, the growth inhibition was recorded under an inverted microscope, after 72h incubation at room temperature.

2.5. Determination of Minimum Fungicidal Concentration (MFC)

Minimum fungicidal concentration (MFC) was determined by transferring a small amount of sample contained in each well of the microtiter plate on which the MIC was calculated, to Sabouraud Dextrose plates (Neogen® Culture Media, USA) using a replicator (BoekelScientific, Pennsylvania, USA). The plates were incubated at 37° C for 24 h. MFC was determined as the lowest concentration of honey at which, no fungal growth was observed. This test was performed only on *C. albicans* strains because the other tested fungi have the potential to form hyphae and spores that the replicator could not efficiently transfer to an agar plate.

2.6. Antagonism Assay

In order to investigate the antifungal activity of bacterial isolates against *P. commune, P. expansum,* and *A. niger*, the antagonism assay was implemented. This method tests the ability of bacteria to inhibit fungal growth as the microorganisms grow together allowing competitive exclusion (Molina-Romero et al., 2017). Potentially fungistatic/fungicidal bacterial strains were grown on Plate Count Agar (PCA) (Neogen® Culture Media, USA) for 24 hours prior to testing. Fungal spore suspension (OD_{530} = 0,3-0,35) was spread on PCA plates using a sterile cotton swab. Afterward, bacterial colonies were placed on a Petri dish in triplicates and the dishes were incubated at room temperature (25°C). Depending on the growth rate of each fungal

strain, conclusive observations were made at 48h for *P. commune*, *P. expansum*, and at 72h for *A. niger*. Results were determined by subtracting the diameter of the bacterial colonies from the diameter of the inhibition zone. Mean values and standard deviation were calculated from triplicates.

3. RESULTS AND DISCUSSION

3.1. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Honey Samples Against *C. albicans 10/20 and C. albicans 351/19*

The MIC assay was implemented, in order to determine the fungistatic activity of honey samples against the *C. albicans* strains. Table 3 summarizes the results regarding both strains.

Table 3. Minimum inhibitory concentrations (%v/v) of honey samples *against C. albicans 10/20 and C. albicans 351/19.*

Honey samples	***C. albicans 10/20***	***C. albicans 351/19***
Manuka **195, 229, 183**	25%	25%
233, 240	25%	50%
210, 244	50%	25%
200, 271, 187, 267, 212, 243, 218, 269, 241	50%	50%

Manuka honey as well as three heather honey samples, suppressed the growth of both strains at 25 % (v/v). Honey samples 233 and 240 were able to inhibit *C. albicans 10/20* growth at 25% (v/v), while samples 210 and 244 halted *C. albicans 351/19* growth at the same concentration. Overall, all samples exhibited antifungal activity against the *C. albicans* strains. Table 4 presents the data regarding the MFC against *C. albicans* strains.

Table 4. Minimum fungicidal concentrations (%v/v) of honey samples against *C. albicans 10/20* and *C. albicans 351/19.*

Honey samples	***C. albicans 10/20***	***C. albicans 351/19***
229, 244, 243, 241, 195, 233	50%	50%
Manuka	≥50%	≥50%
200, 210, 183	>50%	50%
267, 218	50%	>50%
240, 271, 187, 212, 269,	>50%	>50%

Manuka honey exhibited fungicidal activity at a concentration higher than 50% against both strains. Heather honey samples 229, 244, 243, 241, 195, and 233 surpassed Manuka honey activity since they showed fungicidal activity against both strains at 50%. A total of eight (8) out of sixteen (16) honey samples were able to kill at least one of the *C. albicans* strains at a concentration of 50% (v/v).

This is the first study presenting data on the antifungal activity of Greek honeys against *C. albicans* strains. In a recent study, Fernandes et al. (2021), tested heather and chestnut honey from Portugal against *Candida* species and determined MICs at 50% v/v, while MFCs were above 50% v/v. In the same study, Manuka honey used in comparison exerted activity at the same concentration. Concentrations of phenols, flavonoids, and hydrogen peroxide of tested honey samples and Manuka honey were similar. Furthermore, the antifungal activity of Portuguese heather honey against *C. albicans* was previously demonstrated by Feás & Estevinho (2011), who determined MIC at 60% v/v. Our data are in accordance with previous studies on Portuguese honey. However, some of the Greek honey samples, exerted fungistatic activity at 25% v/v and fungicidal activity at 50% v/v, surpassing that of Manuka (with the highest available antimicrobial activity, MGO 1122) and Portuguese honey samples.

On the other hand, Kolayli et al. (2020), implementing an agar diffusion method, observed a lack of inhibitory activity against *C. albicans* of heather and chestnut honey harvested in Türkiye, as well as Manuka honey. Kunčič et al. (2012) reached the same conclusion regarding chestnut honey of Slovenian origin. The discrepancy in the results indicates that the activity of honey could be heavily affected by the implemented method of determining the antimicrobial activity. It is generally accepted that determination of MIC is a more sensitive and quantitatively precise method to study antimicrobial activity compared to agar-well diffusion assay because diffusion rates of active substances might be slower in agar than in broth (Anthimidou & Mossialos, 2013).

3.2. Minimum Inhibitory Concentration (MIC) of honey samples against *P. commune, P. expansum, A. niger*

Microtiter plates of two-fold diluted tested honey and food spoilage fungi were incubated for 72 hours at room temperature and their growth was studied under an inverted microscope. The Table 5 summarizes the MICs of each honey against *P. commune, P. expansum* and *A. niger*.

Table 5. Minimum inhibitory concentrations %v/v) of honey samples against *P. commune*, *P. expansum*, *A. niger*

Samples	*P. commune*	*P. expansum*	*A. niger*
Manuka	50%	50%	50%
200, 267, 210, 212, 229, 244, 243, 241			
183, 218, 269, 195, 233	50%	50%	>50%
178	50%	>50%	50%
240	>50%	50%	>50%
271	>50%	>50%	50%

Manuka honey was able to inhibit the growth of all tested spoilage fungi at 50% concentration. Eight (8) tested honeys, out of which four (4) heather and four (4) Chestnut honey samples, exerted the same activity as Manuka honey.

Penicillium expansum and *Aspergillus niger* were reported to be more sensitive to honey of diverse botanical origin in previous studies (Ahmad et al., 2017; Kunat-Budzyńska et al., 2023; Suhana et al., 2015; Vică et al., 2022, p. 4). Kacániová et al. (2010), implementing agar well diffusion assay, established that Chestnut honey could inhibit, though not completely, *P. expansum* growth at 10% w/v concentration. Suhana et al. (2015) determined the MIC of Manuka honey against *A. niger* at 21% v/v, surpassing the other tested honey samples. Of note, this is the first study to present data on honey antifungal activity against *P. commune*.

3.3. Antifungal activity against *P.commune, P. expansum,* and *A. niger* exerted by bacterial strains

Assessment of antifungal activity exerted by bacterial strains was performed by parallel growth of fungal and bacterial strains on the same growth medium. Examples of the observed inhibition zones around the bacterial colonies are depicted in Figure 2 and their values are presented in Table 6.

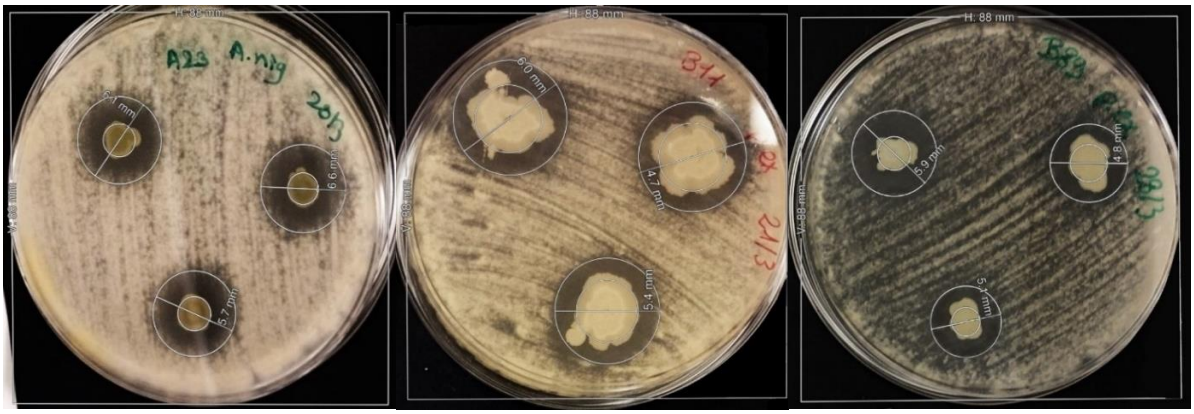

Figure 2. Examples of inhibited fungal growth around bacterial colonies (Left: inhibition of *A. niger* by A23 - *P. fulva*, right: inhibition of *P. expansum* by B11 -*Bacillus sp.*, center: inhibition of *P. expansum* by B89 - *B. safensis*

Table 6. Inhibition zone diameter of bacterial isolates against *P. commune*, *P. expansum*, *A. niger*

Bacterial isolates	***P. commune***	***P. expansum***	***A. niger***
A2	8.4 ± 0.33 mm	17.6 ± 0.53 mm	8.6 ± 0.32mm
A15	8.8 ± 0.57 mm	11.6 ± 1.81 mm	8.5 ± 0.29 mm
A31	8.2 ± 0.34 mm	7.9 ± 0.30 mm	6.8 ± 0.75 mm
A163	4.6 ± 0.21 mm	11.0 ± 0.60 mm	8.0 ± 0.49 mm
B16	4.0 ± 0.42 mm	10.2 ± 0.78 mm	5.2 ± 0.85 mm
B21	8.4 ± 0.62 mm	9.4 ± 1.92mm	9.9 ± 0.74 mm
B89	8.9 ± 0.23 mm	10.5 ± 1.14 mm	9.1 ± 0.54 mm
A20	8.1 ± 1.60 mm	15.1 ± 2.57 mm	9.2 ± 0.50 mm
B11	8.5 ± 0.41 mm	11.1 ± 1.44 mm	7.3 ± 0.34 mm
A28	4.5 ± 1.16 mm	8.2 ± 1.13 mm	5.9 ± 1.20 mm
A23	4.2 ± 0.74 mm	13.1 ± 1.88 mm	12.3 ± 0.74 mm
A138	12.9 ± 2.00 mm	9.4 ± 1.80 mm	5.4 ± 0.20 mm
B7	-	11.5 ± 1.74 mm	6.6 ± 0.28 mm
B120	-	13.0 ± 1.00 mm	5.6 ± 0.34 mm
B34	-	10.2 ± 1.00 mm	9.0 ± 0.65 mm

With the exception of A23-*Pseudomonas fulva*, all tested bacterial strains are *Bacilli*. In previous studies, members of this genus isolated from raw honey were reported to produce *in vitro* a variety of secondary metabolites that could inhibit the growth of other microorganisms in a competitive way. Manns et al. (2012), were able to identify an antifungal peptide produced by *B. thuringiensis* SF361 isolated from honey exerting activity against *Aspergillus*, *Penicillium*, *Byssochlamys*, and *Candida albicans*. Similarly, Xiong et al. (2022), attributed the antifungal activity exerted by two strains of *Bacillus velezensis* to iturin A, a known lipopeptide that inhibits fungal growth. *B. subtilis* and *B. licheniformis* have been the subject of extensive research by Harwood et al., 2018), intending to characterize the synthesis of antifungal non-ribosomally synthesized peptides and polyketides produced by them. Cyclic lipopeptides, such

as surfactin, iturin, pipastatin, and fengysine, applied as antifungal agents for the control of plant diseases, proved to be of outstanding importance (Xiong et al., 2022). Therefore, it is plausible that antifungal activity reported in this study could be attributed to so far unknown secondary metabolites belonging to nonribosomal peptides and /or polyketides. Nevertheless, further research regarding the biosynthetic potential of tested bacterial isolates could elucidate the mechanisms of antifungal activity described in this study.

4. CONCLUSION

In conclusion, this study provides evidence of the antifungal properties of Greek heather and chestnut honeys, alongside certain bacterial isolates, highlighting their potential as antifungal agents.

The results demonstrate that *Penicillium expansum, Penicillium commune, Aspergillus niger,* and *Candida albicans*, are susceptible to the majority of tested honeys, though in a variable way. To the best of our knowledge, this is the first study to investigate the inhibitory effects of honey on *P. commune* growth. Some of the tested honey samples did not only match the antifungal activity of renowned Manuka honey but in certain cases surpassed it, particularly heather honey against *Candida albicans*, exerting fungicidal efficacy at lower concentrations.

Furthermore, our research is the first to examine the *in vitro* antifungal activity of characterized bacterial strains isolated from diverse Greek honey types, against food spoilage fungi. Most isolates were able to inhibit the growth of *Penicillium expansum*, *Penicillium commune,* and *Aspergillus niger*. These findings further support the hypothesis that competitive relationships among microorganisms foster the production of secondary metabolites with antifungal properties.

Given the growing concerns regarding antifungal resistance, our data are important in the search for novel antifungal substances. Future studies should focus on specific bioactive compounds exerting the observed antifungal activity and elucidating their mode of action.

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DECLARATIONS

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Ioanna Boutrou: Investigation, Methodology, Data curation, Writing-Original draft preparation. **Christina Tsadila**: Investigation, Methodology, Data curation. **Chiara Amoroso**: Investigation, Data curation. **Dimitris Mossialos**: Conceptualization, Methodology, Writing-Reviewing and Editing, Supervising

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