Saudi Journal of Biological Sciences 29 (2022) 103462

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Antimicrobial efficacy of extracts of Saudi Arabian desert *Terfezia claveryi* truffles

Hani M.J. Khojah [a,*], Osama B. Abdelhalim [b], Mahmoud A.H. Mostafa [b,c], EL-Sayed E. Habib [d,e]

[a] Department of Clinical and Hospital Pharmacy, College of Pharmacy, Taibah University, Madinah, Saudi Arabia
[b] Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Madinah, Saudi Arabia
[c] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt
[d] Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, Taibah University, Madinah, Saudi Arabia
[e] Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

ARTICLE INFO

Article history:
Received 9 July 2022
Revised 24 August 2022
Accepted 21 September 2022
Available online 27 September 2022

Keywords:
Terfezia claveryi
Truffles
Antimicrobial activity
Anti-biofilm
Saudi Arabia

ABSTRACT

Background: *Terfezia claveryi* truffles are known for their nutritional value and have been considered among traditional treatments for ophthalmic infections and ailments.
Objectives: We sought to investigate the *in vitro* antimicrobial efficacy of several *T. claveryi* extracts from Saudi Arabia. Certain pathogenic fungi and gram-negative and gram-positive bacteria were included.
Methods: Dry extracts were prepared using methanol, ethyl acetate, and distilled water, while the latter was used for preparing fresh extracts. The extracts were microbiologically evaluated through the disc-diffusion agar method; the zones of inhibition of microbial growth were measured post-incubation. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) were determined in Müller-Hinton Broth through the microdilution susceptibility method. anti-biofilm activity was assessed for potent extracts.
Results: Dry extracts showed potent activity (>16-mm inhibition zones) against gram-positive (*Bacillus subtilis* IFO3007 and *Staphylococcus aureus* IFO3060) and gram-negative (*Pseudomonas aeruginosa* IFO3448 and *Escherichia coli* IFO3301) bacteria. The activity against fungi was moderate (12–16-mm inhibition zones) for both *Aspergillus oryzae* IFO4177 and *Candida albicans* IFO0583; there was no activity against *Aspergillus niger* IFO4414 growth. Methanolic extract had the lowest MIC and MBC, exhibiting remarkable activity against *B. subtilis* growth. Fresh extract showed moderate activity against bacterial growth and inactivity against fungal growth. Methanolic extract showed potent anti-biofilm activity (IC_{50}, 2.0 ± 0.18 mg/mL) against *S. aureus*.
Conclusions: *T. claveryi* extracts showed antibacterial effects potentially suitable for clinical application, which warrants further in-depth analysis of their individual isolated compounds.

1. Introduction

In the Arabian region, the desert truffle's common name is Al-Fag'a, while Al-Kam'ah is the classic Arabic name, which means "hidden". Generally, Arabian desert truffles belong to two genera: *Tirmania* sp. and *Terfezia* sp. (Bokhary, 1987). *Tirmania nivea* (white-colored truffle) is called Zubadi or Zubaidi, as a common name, in some Arabian countries. On the other hand, the *Terfezia* sp. is called Ikhlasi or Kholasi and includes *Terfezia boudieri* (black-colored truffles) and *Terfezia claveryi* (brown-colored truffles) (Al-Rawi and Taha, 2010; Bokhary, 1987; Mandeel and Al-Laith, 2007). The Arabian Peninsula represents a well-known source for the latter dark, brown-colored truffles (Al-Rahmah, 2001).

Desert truffles are pale sandy brown, grey or white in color, irregularly spherical, have a slightly spongy texture, have sizes ranging from 1 to 7 cm, and lack a particular smell (Iddison, 2021). They are usually harvested after the rainy season (February

* Corresponding author.
E-mail addresses: hkhojah@taibahu.edu.sa (H.M.J. Khojah), oalbarhamtoshy@taibahu.edu.sa (O.B. Abdelhalim), mamoustafa@taibahu.edu.sa (M.A.H. Mostafa), sayedhabib@mans.edu.eg (EL-Sayed E. Habib).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103462
1319-562X/

to April) in the Arabian Gulf countries (Al-Ruqaie, 2002; Moubasher, 1993). Desert truffles grow in certain areas of Saudi Arabia and the North African deserts, in addition to West Asia and other countries worldwide (Dahham et al., 2018). Some black truffles are highly appreciated worldwide for their pleasant smell and distinctive taste (Mello et al., 2006).

Desert truffles, which are edible mycorrhizal fungi, constitute a traditional food in several cultures because of their medicinal and nutritional properties (Wang and Marcone, 2011). Truffles are considered as very old food that was used instead of meats, and have been highly consumed because of their pleasant aroma and taste (Dundar et al., 2012; Mandeel and Al-Laith, 2007). Their distinct nutritional value appears to be secondary to their rich content of proteins, minerals, vitamins, and unsaturated fatty acids (Patel, 2012). They have been also used for treating ophthalmic infections in traditional medicine (Janakat and Nassar, 2009).

Many compounds have been found in different truffles and some of which have been shown to exhibit different antimicrobial effects (Table 1).

The nutritional value of truffles has been extensively studied (Al-Delaimy, 1977). Unsaturated fatty acids (19.6 %) with a large content of linoleic acid comprise *T. claveryi* (Saddiq and Danial, 2012). *T. claveryi* species truffles of the isolated from Iraq comprise the following: 62 % linoleic acid and 17.6 % protein (Al-Kaisey et al., 1996). A study from Saudi Arabia on *T. claveryi* reported the following composition: 28 % carbohydrate,16 % protein, and 78 % total moisture (Bokhary and Parvez, 1993). It is noteworthy that similar truffle species of different regions may not have similar chemical compositions. These differences of the chemical profiles could be the result of several factors related to the environment including the climatic changes, the amount of rainfall, season, and soil types (Hussain and Al-Ruqaie, 1999).

In addition to the truffles' nutritional value, flavor, and aroma, they are largely underutilized sources of therapeutic compounds. Antimicrobial, anti-cancer, anti-inflammatory, immunosuppressive, and antioxidant effects of truffles have already been reported (Dundar et al., 2012; Hannan et al., 1989; Mandeel and Al-Laith, 2007). *T. claveryi species* was reported useful in the treatment of ophthalmic ailments. Ocular infections are highly linked with bacterial biofilms, as increasing evidence has been reported. A biofilm is a characteristic of bacterial growth when bacteria attach to each other and/or to a surface (Zegans et al., 2002). Hence, ocular infections may be mediated by bacterial biofilms, making bacteria persistent on abiotic surfaces that they attach to, either when implanted eyes or through direct biofilm formation on the ophthalmic biotic surfaces (Saraswathi and Beuerman, 2015; Hou et al., 2012). A boiled water extract of truffle was claimed as an effective remedy for trachoma by the Bedouins; the extracts showed growth inhibition of the etiological agent of trachoma, *Chlamydia trachomatis* (Mandeel and Al-Laith, 2007). Additionally, the antimicrobial activity of several *T. claveryi* extracts (methanolic, aqueous, and partially purified protein) were *in vitro* tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Janakat et al., 2004, 2005). A potent proteinaceous antimicrobial agent against *P. aeruginosa*, that may be useful for treating ophthalmic infections, was reported to exist in the aqueous extract (Janakat et al., 2005).

This research aimed to *in-vitro* study the antimicrobial efficacy of certain extracts of *T. claveryi*, collected from Northern Saudi Arabia, against selected strains of pathogenic fungi and gram-negative and gram-positive bacteria. These microbial strains were not used in previous studies related to the antimicrobial efficacy of truffle extracts.

Table 1
Essential constituents found in truffles.

Active components	Compounds	References
Volatile terpenoids	Carveol; p-cymene; cumene hydroperoxide; guaiene; limonene	Kanchiswamy et al. (2015)
Volatile organic compounds associated with the aroma of the truffles	Dimethyl disulfide; 3-methyl-1-butanol; dimethyl sulfide; ethyl butyrate; 2,3-butanedione; 3-ethyl-5-methylphenol; other aldehydes, alcohols, ketones, organic acids, and sulfurous compounds	Patel (2012), Fratianni et al. (2007), and Culleré et al. (2010)
Phenolic compounds	syringaldehyde; gentisic acid; apigenin; protocatechuic acid; rutin; p-hydroxy syringic acid; benzoic acid; cinnamic acid; catechin p-coumaric acid; ferulic acid	Elsayed et al. (2014), Stanikunaite et al. (2009), and Doğan and Aydın (2013)
Extracellular enzymes	Laccase; cellulase; xylanase; amylase; peroxidase; lipase; catalase	Nadim et al. (2015)
Sterols and triterpenes	5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol; ergosterol; brassicasterol; 3-*O*-β-D-glucopyranosyl-(22E,24R)-ergosta-7,22-dien-5a,6 β-diol; (22*E*,24*R*)-ergosta-5,22-dien-3β-ol; astrapteridone; (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol; astrapteridiol; 3-*epi*-astrapteridiolm; astrahygrone; (22*E*,24*R*)-ergosta-4,6,8 (14),22-tetraen-3-one; 3-*epi*-astrahygrol	Stanikunaite et al. (2008), Segneanu et al. (2021), Harki et al. (1996), and Luo et al. (2011)
Pheromones	5-androstenol (5-α-androst-16-en-3-α-ol)	Wang et al. (2008)
Saturated fatty derivatives	Anandamide (*N*-arachidonoylethanolamine)	Pacioni et al. (2015)
Unsaturated fatty acids	oleic acid; Linoleic acid	Saddiq and Danial (2012) and Al-Kaisey et al. (1996)
Peptides	Proteins; glycoprotein lectins	Janakat et al. (2005) and Hamid and Al-Meani (2021)

2. Materials and methods

2.1. Sample preparation

Truffles were purchased from the seasonal truffle market of the northern part of Saudi Arabia and were kept in clean polyethylene bags during transportation. These fruiting bodies of typical desert *T. claveryi*, locally called Ikhlasi or Kholasi were dark brown in color, small in size, and round in shape (Bokhary, 1987). Distilled water was used for washing a sample of 2 kg thoroughly for 10 times to remove adherent soil. Kitchen sucking papers were then used for blotting the sample that was later cut into small slices.

2.2. Preparation of extracts

Fine pulverized powder of parts of the sample was obtained by drying the parts in a hot oven set at 35–40 °C, until constant weight was achieved after 3 days, followed by milling. The powder was then stored capped at room temperature (25–27 °C). Three parts of the powder, each of approximately 200 g, were used for the extraction through the hot maceration method at 40 °C. Distilled water, ethyl acetate, and methanol were used, in 500 mL each, for the extraction of each part of the powder, respectively. A rotary evaporator was used to filter and concentrate the extracts (Buchi,

USA). The plant extract residues (5 mg each) were re-dissolved in one mL of sterilized distilled water and were stored at −20 °C until use (Saddiq and Danial, 2012).

Another 200 g sample of the fresh slices was homogenized with distilled water (1:3 w/v), at room temperature, using a full-speed household blender for 3 min. The homogenate was kept in a refrigerator overnight, filtered through cheesecloth, and then centrifuged for 15 min at 4,000 rpm. The supernatant was then dried using a rotary evaporator. The dried matter of the aqueous extracts was re-suspended using distilled water and kept at −20 °C until use (Janakat et al., 2004; Viksø-Nielsen et al., 1997).

2.3. Microorganism strains

A panel of standard strains of microorganisms was used for testing the antimicrobial activity of *T. claveryi* extracts. These included two gram-negative bacteria (*P. aeruginosa* IFO3448 and *Escherichia coli* IFO3301), three gram-positive bacteria (*Micrococcus luteus* IFO3232, *Bacillus subtilis* IFO3007, and *S. aureus* IFO3060), and three pathogenic fungi (*Aspergillus niger* IFO4414, *Candida albicans* IFO0583, and *Aspergillus oryzae* IFO4177). The source of these strains was the Institute for Fermentation, (IFO, Osaka, Japan). The microorganism suspensions were adjusted at 10^6 colony-forming units (CFU)/mL.

2.4. The in vitro determination of antimicrobial efficacy

The agar disc-diffusion method was employed to perform primary screening (Clinical and Laboratory Standards Institute, 2005). Separate inoculation of the Müller-Hinton agar medium plates with the studied microorganisms was performed. The measurement of the diameters of inhibition zones was then carried out after incubating the inoculated plates at 37 °C for 24 h. The incubation of the plates of the fungi was performed at 25 °C for 48 h. The antifungal fluconazole and the antibacterial ampicillin were used as positive control (10 µg/disc), while a blank disc, immersed in distilled water, was used as negative control.

The Müller-Hinton broth microdilution susceptibility method was used for the evaluation of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts with high activity against the same microbes from the primary screening (Murray et al., 1995; Wiegand et al., 2008). The extracts then underwent twofold derail dilutions. The lowest concentration that killed the microorganism determined the MBC values, while the lowest concentration that inhibited the growth of the microorganism determined the MIC values (confirmed by subcultures onto sterile Tryptone soya agar plates to verify negative growth). In addition, the extracts with potent efficacy were further challenged for biofilm activity against *S. aureus* IFO3060, which can form a biofilm, in comparison with the antibacterial erythromycin as a reference antibiofilm drug.

Negative control wells were filled with broth only, while 200 mL of bacterial suspension were used to fill sterile 96-well plates. The aerobic incubation of covered plates was then carried out at 37 °C for 24 h. Each well's content was then aspirated, and 250 mL of sterile physiological saline was used to wash the well three times. All non-adherent bacteria were removed by vigorously shaking the plates. The remaining bacteria that were still attached were fixed with methanol, and after 15 min each plate was emptied and left for dryness. Staining of the plates was performed with Gram staining's crystal violet (0.2 mL, 2 %) for 5 min. Following that, running tab water was used to rinse off the excess stain from the plates. After air drying the plates, the adherent cell-bound dye was resolubilized by glacial acetic acid (33 % v/v). Finally, each well's optical density (OD) measurement was carried out at 570 nm (Stepanovic et al., 2000).

3. Results

The preliminary outcomes of the prepared extracts' antimicrobial testing are depicted in Table 2. Most of the prepared extracts showed different inhibition degrees against the tested bacteria. The activity was weak against the pathogenic fungus *C. albicans* and there was no activity against the other fungi. Ethyl acetate, methanolic, and hot aqueous water extracts (dry extract) showed potent activity against the gram-negative *E. coli* and *P. aeruginosa* and the gram-positive *S. aureus* and *B. subtilis* bacteria.

As seen in Table 3, the microbiologically most active extracts in the primary screening were tested for the MIC and MBC against the same microorganisms. The activity demonstrated by the methanolic extract against *B. subtilis* (gram-positive bacterium) was good. Table 4 shows that the same extract has exhibited a more potent anti-biofilm activity (IC_{50}) than the ethyl acetate extract (2.0 ± 0.18 mg/mL and 3.8 ± 0.20 mg/mL, respectively), compared to the IC_{50} of the reference drug erythromycin against the standard strain of the gram-positive *S. aureus* IFO3060 (0.45 ± 0.15 µg/mL).

4. Discussion

Traditionally, most of the studied biological effects of truffles have been related to the antimicrobial activities of desert truffles. For example, the agar-well diffusion assays have demonstrated the antimicrobial activity of *T. boudieri* (Fidan et al., 2022; Hamza et al., 2016). It was suggested that these truffles may have some benefits in the treatment of ophthalmic and dermatologic diseases. In addition, antimicrobial activity was reported for some extracts of *T. claveryi* against *P. aeruginosa* (Gargano et al., 2017).

Truffle extract antimicrobial activity has been also studied based on the solvents used (Dib-Bellahouel and Fortas, 2019; Gargano et al., 2017). Such an activity was reported for methanolic extracts of *Terfezia* sp. against gram-positive bacteria, such as *S. aureus* and *B. subtilis* (Dib-Bellahouel and Fortas, 2019). In addition, methanolic and aqueous extracts of *T. claveryi*, plus aqueous extracts of proteins from the same species, have demonstrated antimicrobial activity against *S. aureus* (Gargano et al., 2017). Furthermore, an inhibition of 40.9 % of *P. aeruginosa* growth was reported by aqueous *T. claveryi* extract, while no activity was reported for methanolic extracts (Dib-Bellahouel and Fortas, 2019).

This research studied the antimicrobial efficacy of extracts of *T. claveryi* obtained by different solvents. The results show that the methanolic, hot aqueous, and ethyl acetate extracts of the dried truffle powder have antimicrobial activity against gram-positive and gram-negative bacteria and fungi (*C. albicans*). The most effective of which was the methanolic extract while the ethyl acetate one may have promising activity on the same microorganisms, in addition to *A. oryzae*. The hot aqueous extract was less active than the methanolic extract. On the other hand, the cold aqueous extract of the dried truffle powder and the cold aqueous extract of fresh truffles have shown the least activity with no effect on *P. aeruginosa* and all the studied fungi.

The methanolic extract in the current study has shown the strongest activity against the gram-positive bacterium *B. subtilis* as reflected by its least MIC and MBC compared with other extracts. Moreover, it exhibited a more potent anti-biofilm activity than the ethyl acetate extract against the gram-positive standard strain of *S. aureus* IFO3060.

This study has also revealed that there could be different antimicrobial compounds in different extracts of *T. claveryi*. The methanolic extract generally contains polar and non-polar active constituents, the ethyl acetate extract contains mainly nonpolar and semipolar compounds, and the aqueous extract contains polar compounds. The precise mechanisms of antimicrobial activities of

Table 2
Antimicrobial activity of tested truffle extracts against a panel of standard strains of gram-positive and gram-negative bacteria and pathogenic fungi.

Extracts	Inhibition zone diameter (mm)*							
	Gram-positive bacteria			Gram-negative bacteria		Fungi		
	S. aureus	*B. subtilis*	*M. luteus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. oryzae*	*A. niger*
Ethyl acetate (dried truffle powder)	22	25	15	20	18	16	12	–
Methanolic (dried truffle powder)	28	30	16	22	20	12	–	–
Hot distilled water (dried truffle powder)	19	20	14	17	15	12	–	–
Cold distilled water (dried truffle powder)	12	15	10	10	–	–	–	–
Cold distilled water (Fresh Truffle)	12	11	8	9	–	–	–	–
Positive controls								
Ampicillin	28	30	25	24	22	NT	NT	NT
Fluconazole	NT	NT	NT	NT	NT	21	22	24

NT, not tested.
* Mean activity values of triplicate results: > 16 mm = strong, 12–16 mm = moderate, 8 to < 12 mm = weak, and < 8 mm = no activity.

Table 3
MICs and MBCs of selected extracts against standard strains of gram-positive and gram-negative bacteria.*

Extracts		Gram-positive		Gram-negative	
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Ethyl acetate (dried truffle powder)	**MIC**	3.5	1.8	4.6	> 5.0
	MBC	6.5	2.5	8.0	NT
Methanolic (dried truffle powder)	**MIC**	1.0	0.5	3.2	> 5.0
	MBC	2.2	1.5	7.2	NT
Hot distilled water (dried truffle powder)	**MIC**	4.2	4.0	> 5.0	> 5.0
	MBC	8.5	8.5	NT	NT
Positive control					
Ampicillin	**MIC**	1.0	0.5	2.5	NT
	MBC	2.5	1.0	5.0	NT

MBC, minimum bactericidal concentration (μg/mL for ampicillin and mg/mL for extracts); ***MIC***, minimum inhibitory concentration (μg/mL for ampicillin and mg/mL for extracts); ***NT***, not tested.
* Mean values of triplicate results.

Table 4
Anti-biofilm activity of selected extracts against *Staphylococcus aureus* IFO3060.

Extracts	Anti-biofilm activity (IC_{50})*
Ethyl acetate (dried truffle powder)	3.8 ± 0.20
Methanolic (dried truffle powder)	2.0 ± 0.18
Reference drug	
Erythromycin	0.45 ± 0.15

IC_{50}, half maximal inhibitory concentration (μg/mL for erythromycin and mg/mL for extracts).
* Mean values ± standard deviation of triplicate results.

the compounds found in truffles have not been reported previously. However, it is proposed that polysaccharides, laccases, lectins, terpenes, phenolic compounds, and anandamide (endocannabinoid) may play the inhibitory role. Bacterial exopolysaccharides can be recognized and eliminated by lectins (Passos da Silva et al., 2019). Moreover, the oxidation of phenolic compounds may be catalyzed by laccases to liberate hydrogen peroxide and superoxide anion radicals, which are both suggested to inhibit pathogenic bacteria (Nadim et al., 2015).

Anandamide, an endocannabinoid, affects quorum sensing and motility, in addition to other specific functions of prokaryotic organisms (Pacioni et al., 2015). The quorum sensing system plays a regulatory role on the expression of many bacterial physiological and virulence factors (Friedman et al., 2019; Lima et al., 2019; Passos da Silva et al., 2017; Xu et al., 2019). Quorum sensing also regulates the formation of biofilms such as the biofilm structure formation, dispersal of biofilm cells, and accumulation of biofilm biomass. It has also been suggested that auto-aggregation in the bacterial system may be promoted by quorum sensing (Deng et al., 2020; Sionov and Steinberg, 2022). Hence, the possible presence of anandamide in the *T. claveryi* methanolic and ethyl acetate extracts may explain the potent activity of these extracts on the biofilm of *S. aureus* IFO3060 (Mukherjee and Bassler, 2019).

On the other hand, biofilms increase the resistance to antimicrobial agents, posing a challenge to human health care (Sharma et al., 2019). Infections associated with biofilms are real therapeutic challenges for microbiologists and clinicians, and the development of novel antimicrobial strategies is an urgent requirement to counteract the resistance to antibiotics in infectious diseases (Saraswathi and Beuerman, 2015). However, more studies are needed because the molecular structures of the proposed bioactive molecules have not been identified by researches that investigated plant extracts with anti-biofilm activity. Studies on natural anti-infective therapy with specific anti-biofilm agents are currently between phase-I and phase-IV clinical trials (Lu et al., 2019).

The antibacterial activity of truffles could also be related to their phenolic compounds. Several studies have shown that polyphenolic compounds are significantly active against *S. aureus*, *B. subtilis*, *E. coli*, *Bacillus cereus*, and *Acetobacter aceti* (Qin et al., 2019). Therefore, the antibacterial efficacy of the methanolic and ethyl acetate truffle extracts might be related to the effects of polyphenols such as p-hydroxy benzoic acid, apigenin, rutin, gentisic acid, ferulic acid, catechin p-coumaric acid, protocatechuic acid, and cinnamic acid (Lima et al., 2019).

The difference between the mechanisms of action by which phenolic compounds affect gram-positive and gram-negative bacteria is not clear. The overall data from earlier studies have demonstrated that phenolic-rich extracts and individual phenolic compounds have an inhibitory effect on the growth of different pathogenic bacteria (Lima et al., 2019; Metsämuuronen and Sirén, 2019).

Terpenoids such as p-cymene, carveol, limonene, guaiene, and cumene hydroperoxide, have also shown selective activity against some gram-positive and gram-negative bacterial and yeast species.

These compounds may interfere with the biofilm development and microbial adhesion on cellular and inert substrates and inhibit the microbial ability to adhere to the inert substrates (Alfonzo et al., 2017; Ceresa et al., 2020; Valdivieso-Ugarte et al., 2019). Therefore, terpenoids may interfere with the first stage of the infectious process.

4.1. Limitations of the study

We were unable to obtain enough truffles for further investigation because they are very expensive: the market price of a kilogram of truffles ranges between €600 and €6000, according to the species (Üstün et al., 2018). In addition, growing truffles is very difficult and often does not bring results as expected. Moreover, finding and collecting truffles naturally is not an easy process (Hall et al., 2008; Üstün et al., 2018). This is because the mycelia of truffles grow underground, and they do not have a stalk or gills (Hall et al., 2008).

5. Conclusions

This study revealed that various extracts of *T. claveryi* have promising antibacterial, antifungal, and antibiofilm effects. Therefore, this species of truffles, in addition to others, could be a rich source of microbiologically active compounds that could help in the treatment of biofilm-resistant bacteria. Further studies on the effects of different extracts on different bacteria and fungi, as well as the effects of individually isolated compounds on these microorganisms, are highly recommended.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The second esteemed author, Prof. Osama B. Abdelhalim has passed away during the preparation of this manuscript. The remaining authors wish to acknowledge his efforts regarding this work and its publication posthumously.

References

Al-Delaimy, K.S., 1977. Protein and amino acid composition of truffle. Can. Inst. Food Sci. Technol. J. 10, 221–222.
Alfonzo, A., Martorana, A., Guarrasi, V., Barbera, M., Gaglio, R., Santulli, A., Settanni, L., Galati, A., Moschetti, G., Francesca, N., 2017. Effect of the lemon essential oils on the safety and sensory quality of salted sardines (Sardina pilchardus Walbaum 1792). Food Control. 73, 1265–1274.
Al-Kaisey, M.T., Hadwan, H.A., Abeed, H.A., Taher, E.J., Dhar, B.L., 1996. Proximate analysis of Iraqi truffle. Mushroom Res. 5, 105–108.
Al-Rahmah, A.N., 2001. Desert and Forest Truffles: Truffles Are a Healing Food [in Arabic]. King Saud University Publications, Riyadh, Saudi Arabia, p. 272.
Al-Rawi, A.A., Taha, A.M., 2010. Chemical study for three Iraqi truffles types [in Arabic]. Anbar J. Agric. Sci. 8, 33–41.
Al-Ruqaie, I.M., 2002. Effect of different treatment processes and preservation methods on the quality of truffles: I. Conventional methods (drying/freezing). Pak. J. Biol. Sci. 5, 1088–1093.
Bokhary, H.A., 1987. Desert truffles "Al-Kamah" of the Kingdom of Saudi Arabia. 1. Occurrence, identification and distribution. Arab Gulf J. Sci. Res. 5, 245–255.
Bokhary, H.A., Parvez, S., 1993. Chemical Composition of Desert Truffles Terfezia claveryi. J. Food Compos. Anal. 6, 285–293.
Ceresa, C., Rinaldi, M., Tessarolo, F., Maniglio, D., Fedeli, E., Tambone, E., Caciagli, P., Banat, I.M., Diaz De Rienzo, M.A., Fracchia, L., 2020. Inhibitory Effects of Lipopeptides and Glycolipids on C. albicans-Staphylococcus spp. Dual-Species Biofilms. Front. Microbiol. 11, 545654.
Clinical and Laboratory Standards Institute, 2005. Performance standards for antimicrobial susceptibility testing. Fifteenth Informational Supplement, M100-S15. CLSI, Wayne, Pennsylvania.
Culleré, L., Ferreira, V., Chevret, B., Venturini, M.E., Sánchez-Gimeno, A.C., Blanco, D., 2010. Characterisation of aroma active compounds in black truffles (Tuber melanosporum) and summer truffles (Tuber aestivum) by gas chromatography–olfactometry. Food Chem. 122, 300–306.
Dahham, S.S., Al-Rawi, S.S., Ibrahim, A.H., Abdul Majid, A.S., Abdul Majid, A.M.S., 2018. Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle Terfezia claveryi. Saudi J. Biol. Sci. 25, 1524–1534.
Deng, Z., Luo, X.M., Liu, J., Wang, H., 2020. Quorum Sensing, Biofilm, and Intestinal Mucosal Barrier: Involvement the Role of Probiotic. Front. Cell. Infect. Microbiol. 10, 538077.
Dib-Bellahouel, S., Fortas, Z., 2019. Antimicrobial Activities of Desert Truffle Extracts and Their Chemical Identification. J. Pharm. Pharmacol. 7, 593–597.
Doğan, H.H., Aydın, S., 2013. Determination of antimicrobial effect, antioxidant activity and phenolic contents of desert truffle in Turkey. Afr. J. Tradit. Complement. Altern. Med. 10, 52–58.
Dundar, A., Faruk Yesil, O., Acay, H., Okumus, V., Ozdemir, S., Yildiz, A., 2012. Antioxidant properties, chemical composition and nutritional value of Terfezia boudieri (Chatin) from Turkey. Food Sci. Technol. Int. 18, 317–328.
Elsayed, E.A., El Enshasy, H., Wadaan, M.A.M., Aziz, R., 2014. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediators Inflamm. 2014, 805841.
Fidan, M., Ali, M.M., Erez, M.E., Cigerci, I.H., Ozdemir, S., Sen, F., 2022. Antioxidant, antimicrobial, cytotoxic and protective effects of truffles. Anal. Biochem. 641, 114566.
Fratianni, F., di Luccia, A., Coppola, R., Nazzaro, F., 2007. Mutagenic and antimutagenic properties of aqueous and ethanolic extracts from fresh and irradiated Tuber aestivum black truffle: A preliminary study. Food Chem. 102, 471–474.
Friedman, L., Smoum, R., Feldman, M., Mechoulam, R., Steinberg, D., 2019. Does the endocannabinoid anandamide affect bacterial quorum sensing, vitality, and motility? Cannabis Cannabinoid Res. 4, 102–109.
Gargano, M.L., Bella, P., Panno, S., Arizza, V., Inguglia, L., Catara, V., Venturella, G., Davino, S., 2017. Antimicrobial Activity of the Extracts of Terfezia Claveryi and Tirmania Pinoyi Against Gram-positive and Gram-negative Bacteria Causal Agent of Diseases in Tomato. Chem. Eng. Trans. 58, 73–78.
Hall, I.R., Brown, G., Zambonelli, A., 2008. Taming the Truffle: the History, Lore, and Science of the Ultimate Mushroom. Timber Press, Portland.
Hamid, L.L., Al-Meani, S.L., 2021. Extraction and Purification of a Lectins from Iraqi Truffle (Terfezia sp.). Egypt. J. Chem. 64 https://ejchem.journals.ekb.eg/article_156898.html.
Hamza, A., Zouari, N., Zouari, S., Jdir, H., Zaidi, S., Gtari, M., Neffati, M., 2016. Nutraceutical potential, antioxidant and antibacterial activities of Terfezia boudieri Chatin, a wild edible desert truffle from Tunisia arid zone. Arab. J. Chem. 9, 383–389.
Hannan, M.A., Al-Dakan, A.A., Aboul-Enein, H.Y., Al-Othaimeen, A.A., 1989. Mutagenic and antimutagenic factor(s) extracted from a desert mushroom using different solvents. Mutagenesis. 4, 111–114.
Harki, E., Klaebe, A., Talou, T., Dargent, R., 1996. Identification and quantification of Tuber melanosporum Vitt. sterols. Steroids. 61, 609–612.
Hou, W., Sun, X., Wang, Z., Zhang, Y., 2012. Biofilm-forming capacity of Staphylococcus epidermidis, Staphylococcus aureus, and Pseudomonas aeruginosa from ocular infections. Invest. Ophthalmol. Vis. Sci. 53, 5624–5631.
Hussain, G., Al-Ruqaie, I.M., 1999. Occurrence, chemical composition, and nutritional value of truffles: an overview. Pak. J. Biol. Sci. 2, 510–514.
Iddison, P. Truffles in Middle Eastern cookery. Emirates Natural History Group (Patron. H.E. Sheikh Nahayan Bin Mubarak Al Nahayan). http://enhg.org/alain/phil/truffle/truffle.htm. (Accessed 20 May 2021).
Janakat, S., Al-Fakhiri, S., Sallal, A.K., 2004. A promising peptide antibiotic from Terfezia claveryi aqueous extract against Staphylococcus aureus in vitro. Phytother. Res. 18, 810–813.
Janakat, S.M., Al-Fakhiri, S.M., Sallal, A.K., 2005. Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle Terfezia claveryi against Pseudomonas aeruginosa. Saudi Med. J. 26, 952–955.
Janakat, S., Nassar, M., 2009. Hepatoprotective Activity of Desert Truffle (Terfezia claveryi) in Comparison with the Effect of Nigella sativa in the Rat. Pak. J. Nutr. 9, 52–56.
Kanchiswamy, C.N., Malnoy, M., Maffei, M.E., 2015. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci. 20, 206–211.
Lima, M.C., Paiva de Sousa, C., Fernandez-Prada, C., Harel, J., Dubreuil, J.D., de Souza, E. L., 2019. A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. Microb. Pathog. 130, 259–270.
Lu, L., Hu, W., Tian, Z., Yuan, D., Yi, G., Zhou, Y., Cheng, Q., Zhu, J., Li, M., 2019. Developing natural products as potential anti-biofilm agents. Chin. Med. 14, 11.
Luo, Q., Zhang, J., Yan, L., Tang, Y., Ding, X., Yang, Z., Sun, Q., 2011. Composition and antioxidant activity of water-soluble polysaccharides from Tuber indicum. J. Med. Food. 14, 1609–1616.
Mandeel, Q.A., Al-Laith, A.A.A., 2007. Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. J. Ethnopharmacol. 110, 118–129.

Mello, A., Murat, C., Bonfante, P., 2006. Truffles: much more than a prized and local fungal delicacy. FEMS Microbiol. Lett. 260, 1–8.
Metsämuuronen, S., Sirén, H., 2019. Bioactive phenolic compounds, metabolism and properties: a review on valuable chemical compounds in Scots pine and Norway spruce. Phytochem. Rev. 18, 623–664.
Moubasher, A.H., 1993. Soil Fungi in Qatar and Other Arab Countries. Qatar University of Qatar, Centre for Scientific and Applied Research, Doha, p. 566.
Mukherjee, S., Bassler, B.L., 2019. Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol. 17, 371–382.
Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., 1995. Manual of Clinical Microbiology. American Society for Microbiology, Washington, District of Columbia.
Nadim, M., Deshaware, S., Saidi, N., Abd-Elhakeem, M., Ojamo, H., Shamekh, S., 2015. Extracellular enzymatic activity of Tuber maculatum and Tuber aestivum Mycelia. Adv. Microbiol. 5, 523–530.
Pacioni, G., Rapino, C., Zarivi, O., Falconi, A., Leonardi, M., Battista, N., Colafarina, S., Sergi, M., Bonfigli, A., Miranda, M., Barsacchi, D., Maccarrone, M., 2015. Truffles contain endocannabinoid metabolic enzymes and anandamide. Phytochemistry. 110, 104–110.
Passos da Silva, D., Schofield, M.C., Parsek, M.R., Tseng, B.S., 2017. An update on the sociomicrobiology of quorum sensing in Gram-negative biofilm development. Pathogens. 6, 51.
Passos da Silva, D., Matwichuk, M.L., Townsend, D.O., Reichhardt, C., Lamba, D., Wozniak, D.J., Parsek, M.R., 2019. The Pseudomonas aeruginosa lectin LecB binds to the exopolysaccharide Psl and stabilizes the biofilm matrix. Nat. Commun. 10, 2183.
Patel, S., 2012. Health and agricultural importance of truffles: a review of current scientific literature. Curr. Trends Biotechnol. Pharm. 6, 15–27.
Qin, F., Yao, L., Lu, C., Li, C., Zhou, Y., Su, C., Chen, B., Shen, Y., 2019. Phenolic composition, antioxidant and antibacterial properties, and in vitro anti-HepG2 cell activities of wild apricot (Armeniaca sibirica L. Lam) kernel skins. Food Chem. Toxicol. 129, 354–364.
Saddiq, A.A., Danial, E.N., 2012. Assessment of phenolic Content, Free Radical-Scavenging Capacity and antimicrobial Activities of Truffle claveryi. Wulfenia J. 19, 403–422.
Saraswathi, P., Beuerman, R.W., 2015. Corneal biofilms: from planktonic to microcolony formation in an experimental keratitis infection with Pseudomonas aeruginosa. Ocul. Surf. 13, 331–345.
Segneanu, A.-E., Cepan, M., Bobica, A., Stanusoiu, I., Dragomir, I.C., Parau, A., Grozescu, I., 2021. Chemical Screening of Metabolites Profile from Romanian Tuber spp. Plants. 10 (3), 540.
Sharma, D., Misba, L., Khan, A.U., 2019. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob. Resist. Infect. Control 8, 76.
Sionov, R.V., Steinberg, D., 2022. Targeting the Holy Triangle of Quorum Sensing, Biofilm Formation, and Antibiotic Resistance in Pathogenic Bacteria. Microorg. 10, 1239.
Stanikunaite, R., Radwan, M.M., Trappe, J.M., Fronczek, F., Ross, S.A., 2008. Lanostane-type triterpenes from the mushroom Astraeus pteridis with antituberculosis activity. J. Nat. Prod. 71, 2077–2079.
Stanikunaite, R., Khan, S.I., Trappe, J.M., Ross, S.A., 2009. Cyclooxygenase-2 inhibitory and antioxidant compounds from the truffle Elaphomyces granulatus. Phytother. Res. 23, 575–578.
Stepanovic, S., Vukovic, D., Dakic, I., Savic, B., Svabic-Vlahovic, M., 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods. 40, 175–179.
Üstün, N., Bulam, S., Peksen, A., 2018. Biochemical properties, biological activities and usage of truffles. International Congress on Engineering and Life Science. ICELIS, Kastamonu, Turkey.
Valdivieso-Ugarte, M., Gomez-Llorente, C., Plaza-Díaz, J., Gil, Á., 2019. Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. Nutrients. 11.
Viksø-Nielsen, A., Christensen, T.M.I.E., Bojko, M., Marcussen, J., 1997. Purification and characterization of β-amylase from leaves of potato (Solanum tuberosum). Physiol. Plant. 99, 190–196.
Wang, G., Li, Y.Y., Li, D.S., Tang, Y.J., 2008. Determination of 5alpha-androst-16-en-3alpha-ol in truffle fermentation broth by solid-phase extraction coupled with gas chromatography-flame ionization detector/electron impact mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 870, 209–215.
Wang, S., Marcone, M.F., 2011. The biochemistry and biological properties of the world's most expensive underground edible mushroom: truffles. Food Res. Int. 44, 2567–2581.
Wiegand, I., Hilpert, K., Hancock, R.E.W., 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 3, 163–175.
Xu, Z., Xie, J., Soteyome, T., Peters, B.M., Shirtliff, M.E., Liu, J., Harro, J.M., 2019. Polymicrobial interaction and biofilms between Staphylococcus aureus and Pseudomonas aeruginosa: an underestimated concern in food safety. Curr. Opin. Food Sci. 26, 57–64.
Zegans, M.E., Becker, H.I., Budzik, J., O'Toole, G., 2002. The role of bacterial biofilms in ocular infections. DNA Cell Biol. 21, 415–420.