

Antimicrobial Activities of Desert Truffle Extracts and Their Chemical Identification

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Abstract: Desert truffles are edible mushrooms and they belong to groupe of ascomycetes. In Algeria, we find them in arid and semiarid area. They are represented by two most genres: *Tirmania* (white terfez) and *Terfezia* (red terfez). The aim of our work is to present extraction technics of the bioactive substances from gleba (ascoms) of *Tirmania pinoyi* (Maire) Malençon and from the culture filtrate of mycelium of *Terfezia clavaryi* Chatin. Also, we present antifungal and antibacterial activity of these substances *in vitro* and their identification. There is antifungal activity of different extracts on solid medium, *in vitro*, against species of *Aspergillus*, *Fusarium*, *Gliocladium*, *Candida*. Also, there is antibacterial activity against species of *Staphylococcus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Escherichia*, and *Salmonella*. Results show that extracts inhibition varies according to microbial tested species. Bioactive substances of the different extracts were analyzed by GC (gas chromatography)-mass, RMN (Nuclear Magnetic Resonance) and infrared in order to determine the chemical identity. Results show that these substances contain pyrazines and cyclic compounds like quinones.

Key words: Desert truffle, antimicrobial activity, bioactive substances, chemical identification.

1. Introduction

Desert truffles are hypogeous ascomycetes living in mycorrhizal association with herbaceous plants including Cistaceae in the arid and semiarid zones of Algeria. They are mostly represented by the genera *Tirmania* (white terfez) and *Terfezia* (red terfez) [1, 2].

The terfez is appreciated for their nutritional value but also for their properties in traditional medicine.

The aim of our work is to obtain extracts from ascoms (gleba) and mycelial culture filtrate of terfez to test their biological activity on bacterial and fungal species, and to analyze bioactive substances by chromatographic and spectroscopic methods for their identification.

2. Materials and Methods

2.1 Desert Truffle Species

The two species of terfez studied are *Tirmania*

pinoyi (Maire) Malençon and *Terfezia clavaryi* Chatin. Ascoms of the first species came from the arid zones of Algeria. The mycelial strain of the second species came from the Laboratory of Biology of Microorganisms and Biotechnology of Oran.

2.2 Fungal Species Tested

Filamentous fungi were used: *Gliocladium roseum*, *Fusarium oxysporum* f.sp. *albedinis*, *Aspergillus niger* ATCC 16404 and the yeast, *Candida albicans*.

2.3 Bacterial Species Tested

Gram+ bacterial strains tested were: *Bacillus subtilis*, *Streptococcus* ATCC 29212, *Staphylococcus aureus* and *Enterococcus faecalis*. Gram- bacterial strains tested were: *Escherichia coli* ATCC 25922 and *Salmonella typhimurium*.

2.4 Fungal Cultures

The 4 fungal strains are seeded on PDA (potato dextrose agar) medium in Petri dishes. The strain of *Candida albicans* is seeded by the streaking method

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while the filamentous fungi are seeded by mycelial implant (1 cm²). The Petri dishes are incubated at 25 °C for 7 days.

2.5 Bacterial Cultures

The bacterial strains are seeded by streaking method on Mueller-Hinton medium, in Petri dish. The cultures are incubated at 37 °C for 24 to 48 hours.

2.6 Extraction of Bioactive Substances

2.6.1 Extraction from the Gleba of *Tirmania pinoyi*

Fragments of gleba was taken from dried ascoms of *Tirmania pinoyi* and placed in the Soxhlet with ethyl acetate and heated for 3 h. The solvent is then removed with Rotavapor and the extract is thus recovered [3].

2.6.2 Extraction from the Culture Filtrate of *Terfezia claveryi*

After 60 days of incubation, the mycelial cultures of *Terfezia claveryi*, in 1% malt liquid medium, are filtered. The extraction consists of mixing 1 L of the culture filtrate with the ethyl acetate in a separating funnel. After double extraction, the organic phase is recovered in a Becher containing anhydrous sodium sulphate. It is filtered and concentrated in a Rotavapor. The extract is thus recovered.

2.7 Activity Tests of Extracts on the Growth of Fungal and Bacterial Species

2.7.1 Antibacterial Test

The bacterial inoculum is seeded on Mueller-Hinton medium in Petri dishes. In each Petri dish, 3 discs of 6mm in diameter are impregnated with the extract and are deposited on the surface of the seeded medium. Discs deposited in the control Petri dishes are impregnated with ethyl acetate.

2.7.2 Antifungal Tests

1 mL of the sporal suspension of the filamentous fungus is deposited in each Petri dish. The PDA medium is then poured and homogenized with the inoculum. After solidification of the medium, 3 wells

of 8 mm in diameter are made in each Petri dish. Each well is filled with the extract. The wells of the control Petri dishes are filled with the solvent only (ethyle acetate). For the yeast test, the Mueller-Hinton medium replaced the PDA medium. In all cases, the Petri dishes are sealed with adhesive tape and then incubated at 25 °C for 7 days.

2.7.3 Measurement of Microbial Growth

The sensitivity of the microbial species tested is determined by measuring the diameters of the inhibition zones around the discs or wells, in two perpendicular directions.

2.8 Identification of Bioactive Substances

We were interested only in the extract of the gleba of *Tirmania pinoyi*. This extract is fractionated by GC (gas chromatography) coupled to the mass. The other part is fractionated by CC (column chromatography) and then analyzed by spectroscopic methods: infra-red, ¹H NMR and ¹³C NMR.

2.8.1 Chromatographic Methods

(1) CC

It is a technique based on adsorption phenomena. The solid phase is silica gel. The mobile phase (eluent) is a mixture of dichloromethane and methanol.

The column is filled at 2/3 with silica dissolved in dichloromethane. The extract is deposited at the top of the gel and then eluent volumes of different proportions are poured. The fractions are regularly recovered in tubes below the column.

In order to follow the progression of the separation, thin layer chromatography (CCM plates) is carried out. The eluent in this chromatography is a mixture of dichloromethane/methanol.

(2) GC Coupled to Mass

The principle of GC is separation between two phases. One of these phases is a stationary liquid uniformly distributed as a thin film on an inert solid with a large specific surface area, while the other is a mobile gas that flows through the stationary assembly.

The mass spectrum is coupled to the GC. The latter

provides information on the molecular mass, the formula and the disposition of specific groups within the molecule.

2.8.2 Spectroscopic Methods

(1) Infrared Spectroscopy

The technique consists of dissolving the fraction in potassium bromide (KBr). This mixture is introduced into the pelletizer then is subjected to a mass of $10 \times 1,000$ kg; we obtain a pellet of 13 mm in diameter. The pellet is placed on a sample holder and then introduced into the infrared apparatus; the vibration frequencies are expressed in cm^{-1} .

(2) Nuclear Magnetic Resonance Spectroscopy (NMR)

The proton and carbon 13 NMR spectra were recorded on a “Bruker AC300” apparatus. The technique consists of dissolving an aliquot of the fraction in deuteriochloroform, the solution obtained is

transferred into an NMR (nuclear magnetic resonance) tube which is placed in the apparatus. The recorder notes the peaks of the compounds present in the fraction. Proton NMR gives peaks that reflect methyl chemical shifts.

In addition, the carbon 13 NMR makes it possible to eliminate all the carbon-proton couplings so that each carbon gives rise to a single peak on the spectrum.

3. Results and Discussion

The extract of the culture filtrate of *Terfezia clavervyi* inhibits the growth of *Gliocladium roseum* (Fig. 1). It also inhibits *Candida albicans* with an inhibition zone between 11 and 23 mm.

The strain of *Fusarium oxysporum* f.sp. *albedinis* is strongly inhibited by *Tirmania pinoyi* extract after 4 days of culture (Fig. 2). This extract also inhibits the

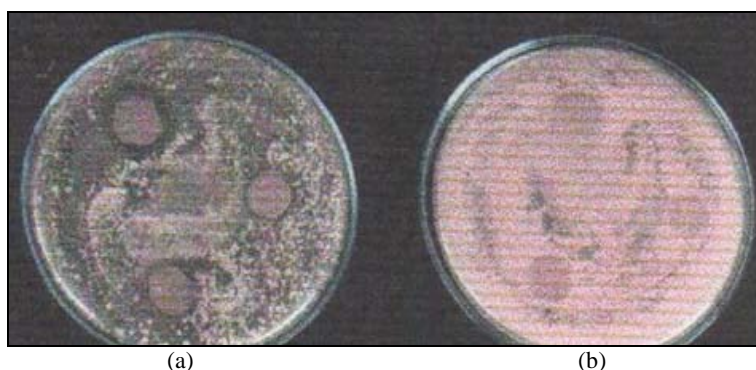


Fig. 1 The extract of the culture filtrate of *Terfezia clavervyi* inhibits the growth of *Gliocladium roseum*. (a): tested, (b): control.

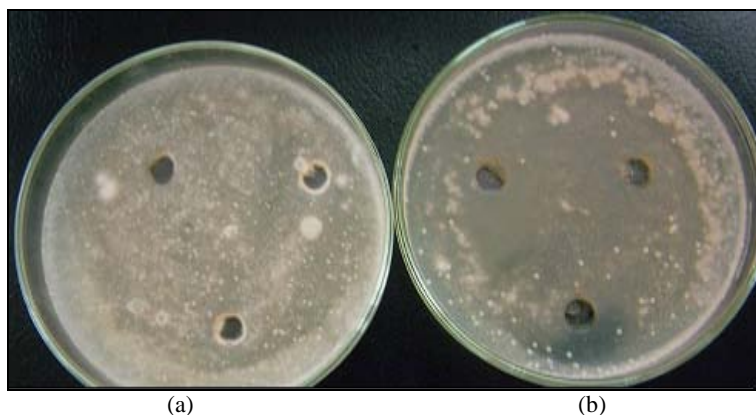


Fig. 2 The extract of *Terfezia clavervyi* culture filtrate inhibits the growth of *Fusarium oxysporum* f.sp. *albedinis*. (a): control, (b): tested.

mycelial growth of *Aspergillus niger* ATCC 16404 from the 7th day.

The extract of the culture filtrate from *Terfezia claveryi* inhibits the growth of *Staphylococcus aureus* after 24 h of incubation (Fig. 3). The inhibition zone is 10.8 mm.

Tirmania pinoyi extract inhibits in ascending order: *Streptococcus* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella typhimurium*, *Enterococcus faecalis* (Fig. 4) and *Bacillus subtilis*. The diameters of inhibition zones vary from 10 to 17 mm.

These results are similar to those obtained by several researchers [3-7].

Our results show that the extracts are antibacterial but also antifungal. This phenomenon is rare because in general the antibacterial substances are inactive on

fungi because of the differences of structure, organization and chemical composition of the prokaryotic and eukaryotic walls [8].

The GC-mass of the crude extract of the dried gleba of *Tirmania pinoyi* revealed the presence of the pyrazines according to their retention times at 5.98 and the molecular weight at 108 (Fig. 5).

Pyrazines are aromatic and heterocyclic organic compounds. Their derivatives are known for their antibiotic activity [9, 10].

Fractionation of this extract by CC and CCM yielded several fractions. After analyzing one of the fractions, we detected the presence of quinone-like benzene rings by IR ($1,103.08\text{ cm}^{-1}$), ^1H NMR (7.6 ppm) and ^{13}C NMR (130 ppm). Quinones are oxygenated aromatic compounds that are biologically very active [11].

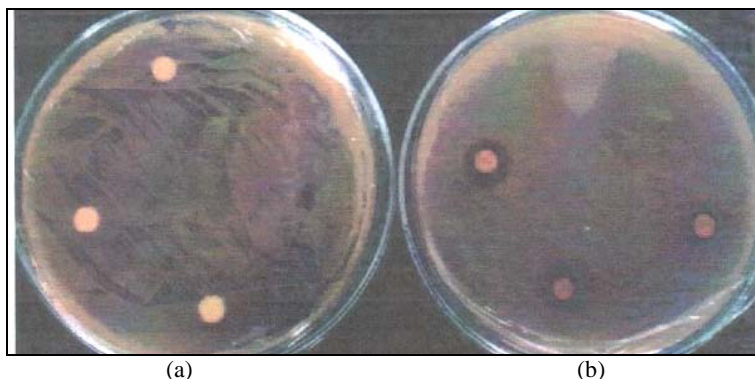


Fig. 3 The extract of *Terfezia claveryi* culture filtrate inhibits the growth of *Staphylococcus aureus*. (a): control, (b): tested.

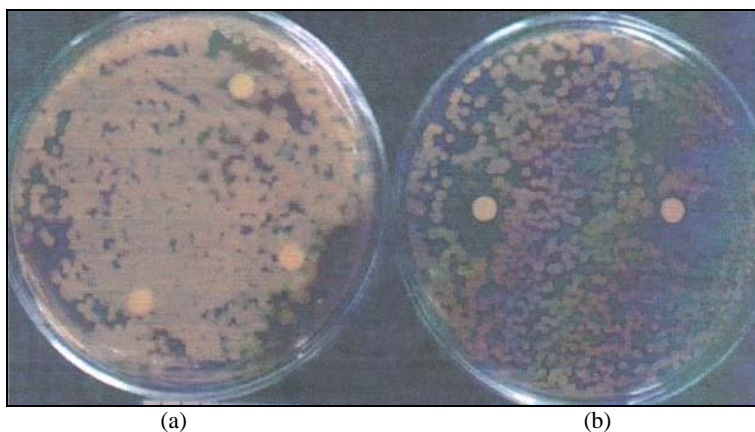


Fig. 4 The extract of the gleba of *Tirmania pinoyi* inhibits the growth of *Enterococcus faecalis*. (a): control, (b): tested.

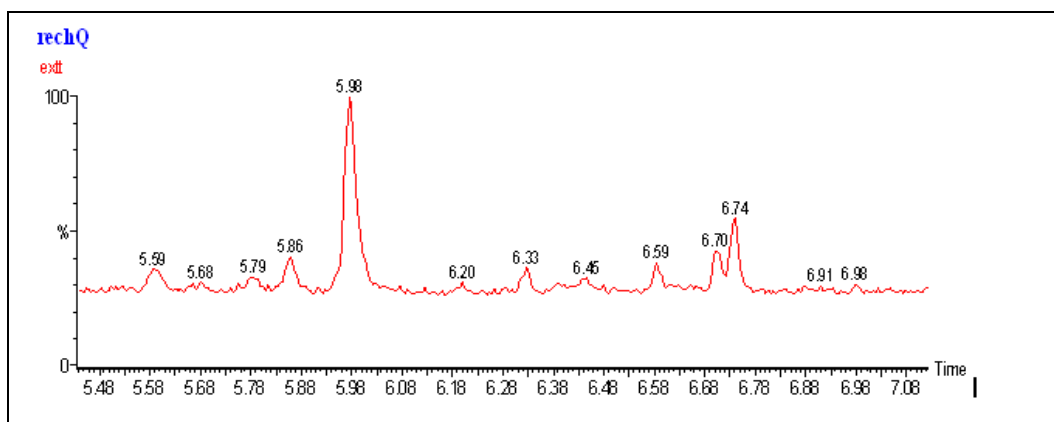


Fig. 5 The chromatogram (a part) of the crude extract of *Tirmania pinyi*.

4. Conclusions

Our work shows that the two terfez species studied *Terfezia clavervyi* and *Tirmania pinoyi*, possess substances with antibacterial and antifungal activities. Bioactive substances appear to be present in the gleba and mycelial terfez culture medium.

Chromatographic and spectroscopic analysis of the gleba extract of *Tirmania pinoyi* revealed the presence of pyrazines and benzene rings resembling those of quinones. Derivatives of pyrazines or quinones are known for their active biological power.

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