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Full Length Research Paper

Antibacterial activity of various fractions of ethyl acetate extract from the desert truffle, *Tirmania pinoyi,* preliminarily analyzed by gas chromatography-mass spectrometry (GC-MS)

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The antibacterial activity of various fractions of ethyl acetate extract isolated from edible fungi, *Tirmania pinoyi* (Maire) Malençon, growing in Algeria, was investigated. Extraction was done by the Soxhlet and the fractions obtained were purified with silica-gel column. Two fractions of ethyl acetate extract were tested against the bacteria *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 14028 and *Enterococcus* sp. ATCC 29212 using the agar diffusion technique. These fractions were preliminary analyzed by gas chromatography-mass spectrometry (GC-MS). The results show inhibition zones, with diameters between10 and 22 mm, against *B. subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The fractions were not effective against the other investigated bacteria. The GC-MS showed the presence of several products, some of them have antibacterial activity in the literature.

Key words: Edible fungi, desert truffle, *Tirmania pinoyi*, antibacterial activity, gas chromatography-mass spectrometry (GC-MS), ascocarps.

INTRODUCTION

Tirmania pinoyi is an edible fungi belonging to desert truffles. Desert truffles are hypogeous ascomycetes and mycorrhizal fungi forming association with roots of *Helianthemum* sp. (Mandeel and Al-Laith, 2007). They are socio-economically important fungi. They are consumed in North Africa (Algeria, Morocco, Tunisia and Egypt) and in the Middle East (Saudi Arabia, Kuwait, Iraq, Iran, Lebanon, Syria and Jordon). The truffle of the desert is not so strongly flavored when compared with the European truffles (Al-Sheikh and Trappe, 1983); nevertheless, it is a type of mushrooms highly prized for its unique musky flavor (Omer et al., 1994).

Popularity of desert truffles is due to their nutritional value and delicious taste. Several studies on their chemical composition have shown that they are rich in proteins, amino acids, fiber, fatty acids, minerals and carbohydrates (Ahmed et al., 1981; Al- Naama et al., 1988; Bokhary et al., 1987, 1989; Bokhary and Parvez, 1993; Dabbour and Takruri, 2002; Hussain and Al-Ruqaie, 1999; Murcia et al., 2003; Yildiz et al., 2005). The protein content, which averages 20% of the dry weight in desert truffles, is significantly higher than in most vegetables and other fungi, as a consequence, the consumption of these truffles is recommended (Murcia et al., 2003).

Desert truffles comprise a vast unexploited source of therapeutic compounds with anti-inflammatory, immune-suppressor, antimutagenic and anticarcinogenic characteristics (Hannan et al., 1989), and antioxidant properties (Al-Laith, 2010; Murcia et al., 2002; Pervez-Gilabert et al., 2005). Also, the presence of enzymes with great interest was found in the ascocarps of some desert truffle (Pervez-Gilabert et al., 2005).

A promising antibiotic and antimicrobial activity has been detected in desert truffles (Chellal and Lukasova, 1995; Janakat et al., 2004, 2005; Rougieux, 1963). All these findings make desert truffles very remarkable. The

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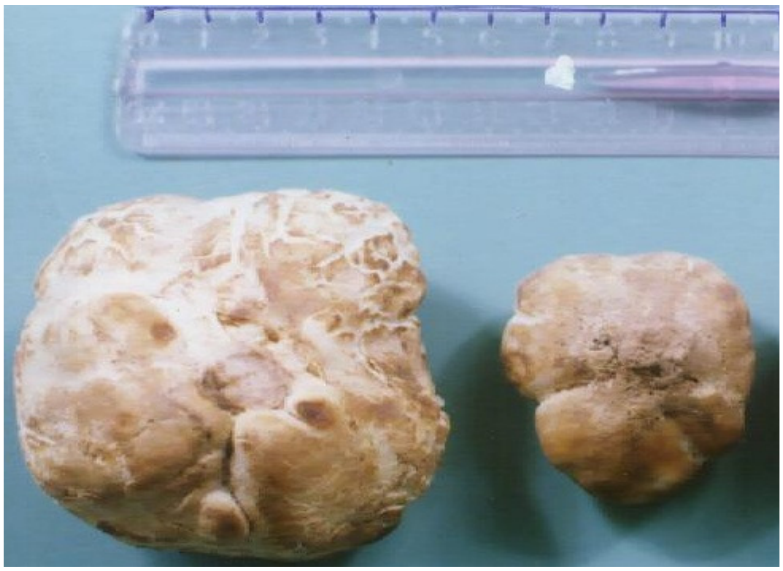

Figure 1. Ascocarps of the desert truffle, *T. pinoyi* (Maire) Malençon.

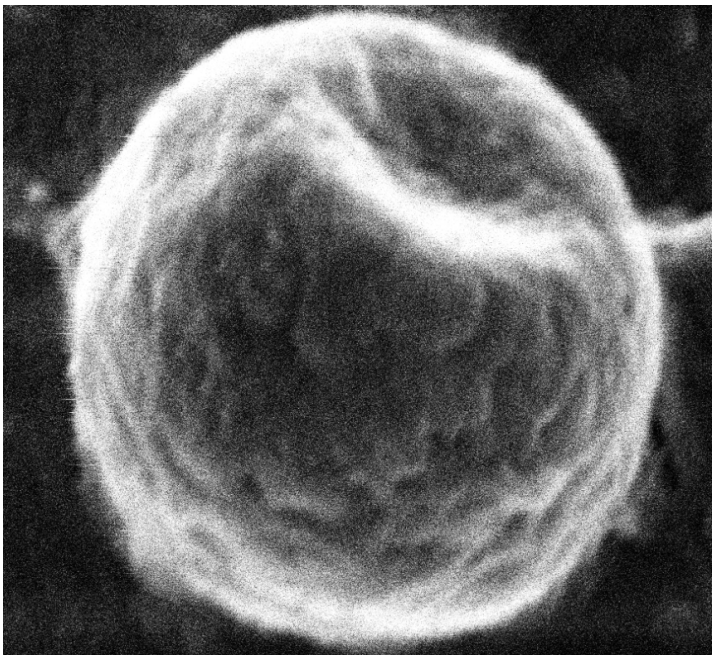

Figure 2. Ascospore of *T. pinoyi* (Maire) Malençon under scanning electron microscopy (x5000).

aim of this study was to investigate the antibacterial activity of products extracted from the dried ascocarps of desert edible truffle, *T. pinoyi* (Maire) Malençon, from South of Algeria.

MATERIALS AND METHODS

Fruiting bodies of desert truffles were collected from the south of Algeria during the month of February (Figure 1). When received, truffle samples were washed and stored at room temperature. They were intact and showed no sign of spoilage. The samples were irregular spherical in shape. The color of flesh was light creamy. Superficially, they were white. When electronic microscopy was used, the spherical shape and smooth surface of ascospores showed that they were those of *T. pinoyi* (Maire) Malençon (Fortas and Chevalier, 1992) (Figure 2). Fruiting bodies were cleaned of soil and were air dried under the sun until constant weight.

The bacterial strains, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 14028 and *Enterococcus* ATCC

Table 1. Percentages of solvent.

Dichloromethane (%)	100	90	80	70	60	0
Methanol (%)	0	10	20	30	40	100

29212 were used for the antibacterial investigations. Bacteria were obtained from the culture collection maintained at Pasteur Institute (France).

Preparation of the extract

Dried ascocarps of the fungi, *T. pinoyi* were peeled, sliced and grinded to uniform powder. 1000 ml of ethyl acetate was placed in a Soxhlet apparatus with 70 g of the powder of the ascocarps. After 3 h, the extract was concentrated using a rotary evaporator (Rotavapor) (Fortas and Belahouel-Dib, 2007).

Purification of the extract by column chromatography and thin layer chromatography (separation of fractions)

A column of 40 cm length and 2 cm diameter with 30 g of silica gel were used; the extract was deposed in the top of the silica gel and volumes of 100 ml mixture of dichloromethane/methanol in different proportions are poured (Table 1) (Fortas and Belahouel- Dib, 2007).

In order to recover fractions, test tubes were placed under column; progress of separation is followed by silica gel plates TLC (thin layer chromatography), using the mixture dichloro-methane/methanol (4/1, v:v) as eluent and the para-anysaldehyde as revelator.

Antibacterial activity

Antibacterial activity of the extract of *T. pinoyi* was tested against strains of Gram positive and negative bacteria by the disc agar diffusion method (Berghe and Vlietinck, 1991; Cappuccino and Sherman, 1998).

Mueller-Hinton agar medium were poured into the plates to uniform depth of 4 mm and allowed to solidify. The microbial suspensions at 10^8cfu/ ml were streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. The discs used were Whatman No 1 papers, 6 mm in diameter. Aliquots of the fractions of ethyl acetate extract were impregnated on filter paper discs, which were then aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37°C for 24 h. Inhibition zones, including the diameter of the discs, were measured (the diameters of inhibition zones were measured in both perpendicular directions around the disc). Control discs were impregnated with aliquots of the solvent ethyl acetate.

Gas chromatography-mass spectrometry (GC-MS)

Mass spectrometry provides information about the molecular mass, form and arrangement of specific groups within the molecule. GC was used (Trace 2000) with detector Ion Trap (Polaris ®).

Gas chromatograph

Column: HP5 MS, 60 m x 0.25 mm x 0.25 µm; Oven program: 60°C for 3 min, then 25°C/min to 150°C with no hold time, 10°C/min to 240°C and hold for 5 min; Volume of injection: 1 µl; Injector temperature: 180°C split less; Gas: Helium- 1.5 ml/min.

Mass spectrometer

Mass range: 40 to 680 UM; Multiplier delay: 6 min; Filament: 20 µamps; Ion trap; Temperature: 250°C.

RESULTS AND DISCUSSION

Fractions of ethyl acetate extract of *T. pinoyi*

The silica-gel column chromatography separated the ethyl acetate extract of *T. pinoyi* into three fractions which was revealed by TLC plates with p-anisaldehyde. Attention was given only to two fractions because of their high concentration in the extract. They were named fraction A (Rf = 0.3) and fraction B (Rf = 0.1).

Antibacterial activity of fractions of ethyl acetate extract

The two fractions isolated from the extract of the dried ascocarps of *T. pinoyi* were tested on the bacterial strains. *In vitro* antibacterial activity of the fractions A and B tested against *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633 is shown in Figure 3. The diameters of inhibition zones after a culture of 24 h are shown in Table 2 and Figure 5.

Both fractions of the extract inhibited growth of only two bacterial strains: *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633. These results confirm antibacterial activity of desert truffles as cited in the literature (Rougieux, 1963; Bokhary et al., 1987, Chellal and Lukasova, 1995; Janakat et al., 2004, 2005).

GC-MS spectra of fractions A and B

The GC- MS spectrum of fraction A revealed products at several times: 4.17, 7.15, 8.84, 17.83, and 20.10. We could identify just the product at 8.837. It was derived from the family of pyrazines. It was pyrazine, 3-ethyl-2,5-dimethyl; its molecular ion peak was 135 (Figures 4 and 5).

The GC-MS spectrum of fraction B revealed several products unknown: 3.35, 3.43, 7.26, 7.98, 10.30, 11.89, 12.45, 12.82, 14.22 and 14.92. We could identify just the product of about 10.256. This product is derived from the family of pyrazines: 1-(6-methyl-2pyrazine)-3 methyl-1-butanol. Its molecular ion peak is 124 (Figures 6 and 7).

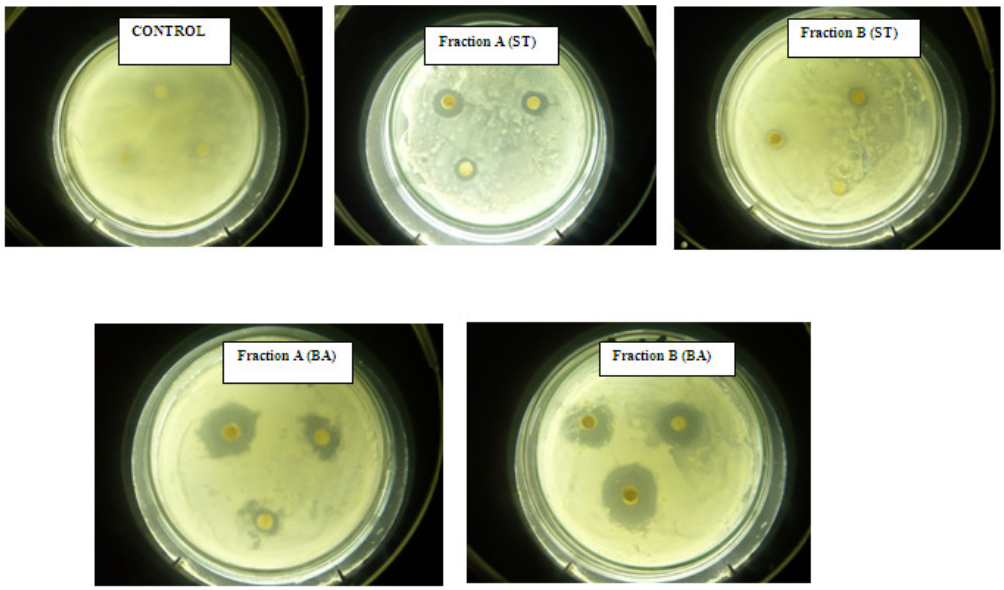

Figure 3. *In vitro* antibacterial activity of the fractions A and B tested against *S. aureus* ATCC 6538 (ST) and *B. subtilis* ATCC 6633 (BA).

Table 2. Diameters of inhibition zones of bacterial growth in the presence of fractions A and B after 24 h (at 37°C) on Mueller and Hinton medium.

Diameter of inhibition zone / Bacterial strain (mm)	Fraction A	Fraction B	Control	Inhibition
S. aureus ATCC 6538	13	10	6	+
B. subtilis ATCC 6633	17	22	6	+
E. coli ATCC 25922	6	6	6	-
P. aeruginosa ATCC 14028	6	6	6	-
Enterococcus sp. ATCC 29212	6	6	6	-

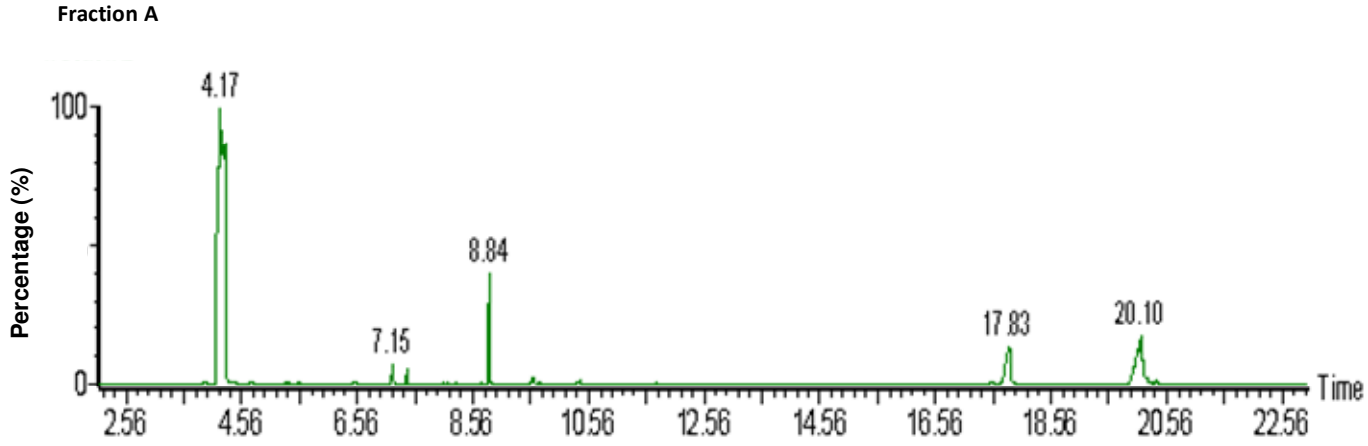

Figure 4. Chromatogram of fraction A.

From the results of GC-MS spectra, the fractions A and B of the ethyl acetate extract of *T. pinoyi* contained several products. We could identify just pyrazines. The pyrazines are organic and heterocyclic compounds, studied for their

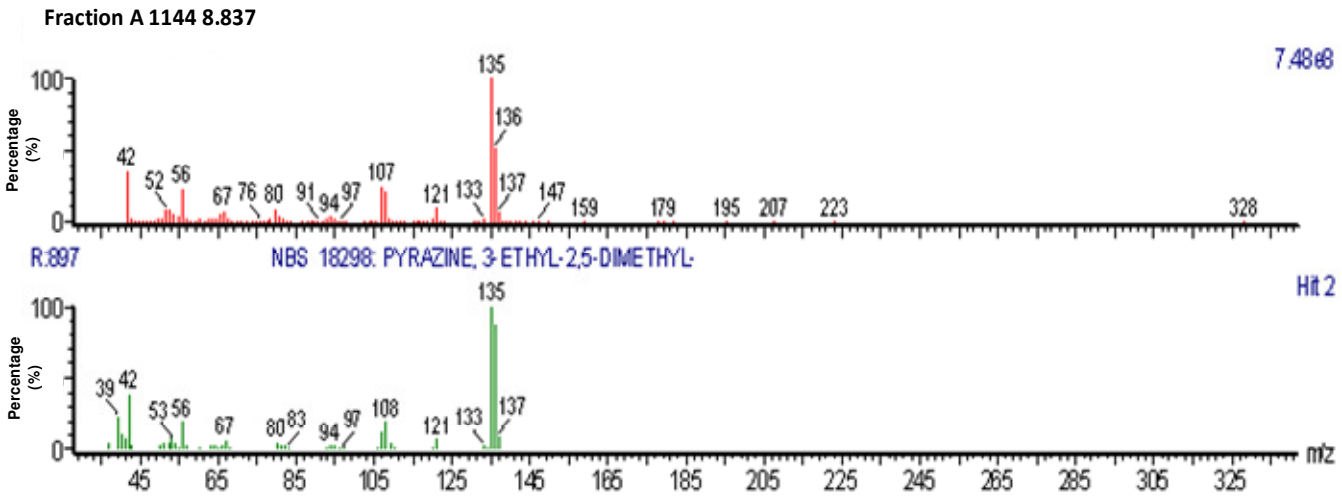

Figure 5. Mass spectrum of fraction A.

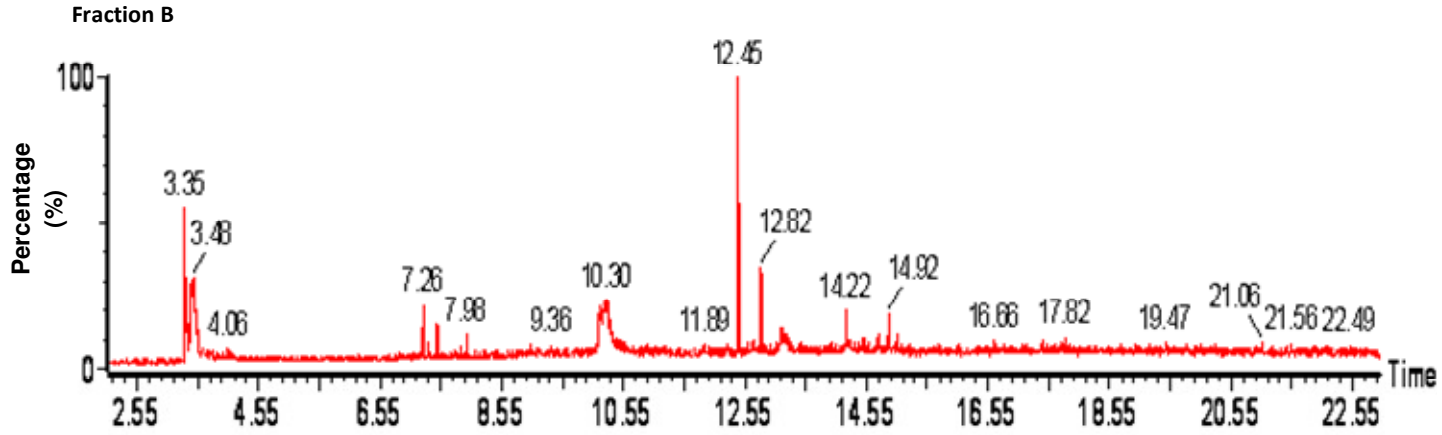

Figure 6. Chromatogram of fraction B

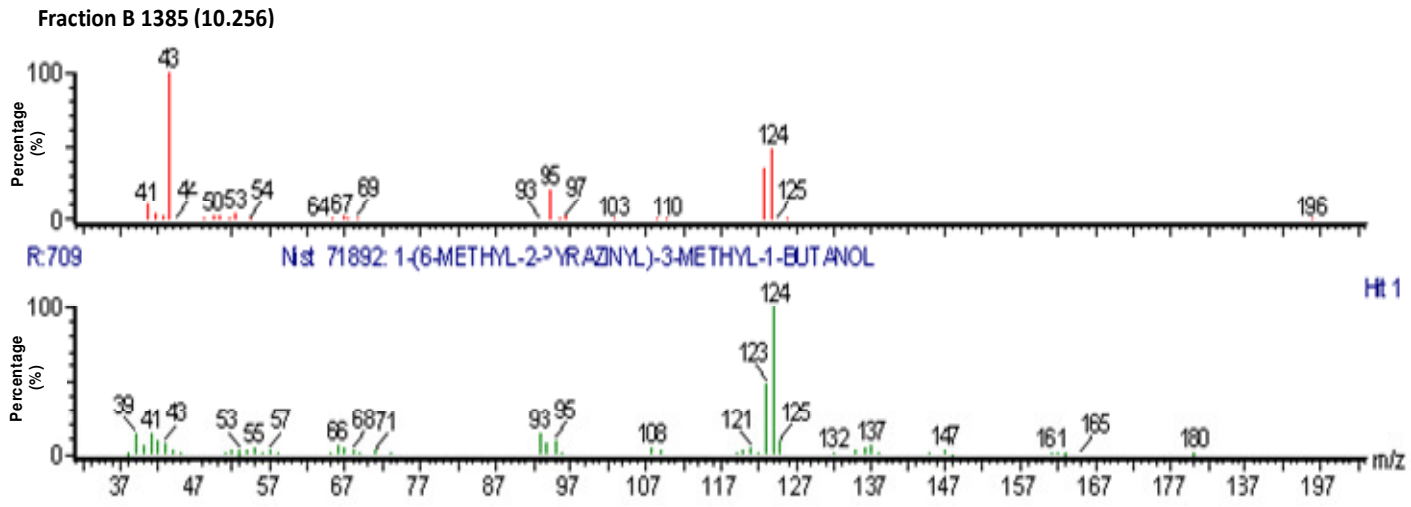

Figure 7. Mass spectrum of fraction B.

antibacterial activity (Dolezal et al., 2003; Bonde and Gaikwad, 2004; Foks et al., 2005). It was also shown that they have a very good anti-mycobacterium activity (Dolezal et al., 2008). Pyrazines derivatives possess other biological properties: hypnotics, sedatives, herbicides, pesticides, etc. (Ohta et al., 1997).

Conclusion

It was found in this preliminary study that fractions of the ethyl acetate extract of the desert edible truffle, *T. pinoyi* contain products which inhibit growth of *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633. *P. aeruginosa* ATCC 14028, *E. coli* ATCC 25922 and *Enterococcus* sp. ATCC 29212 were not inhibited. GC-MS revealed the presence of several products; we could identify just pyrazines which have antibacterial activity in literature.

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