

Chemical Composition, Fatty Acids, Total Phenolics and Antioxidant Activity of the Desert Truffle *Terfezia Boudieri* Chatin in the Northern Region of Saudi Arabia

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Received: 13 April 2024 | Revised: 29 April 2024 | Accepted: 8 May 2024

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ABSTRACT

Terfezia boudieri Chatin (*T. boudieri*), which belongs to the *Terfeziaceae* family, is a desert truffle growing naturally from mycorrhizal relationships with plants that inhabit the slightly moist sandy soils of some desert or semi-desert regions. The chemical examination of the methanolic extract of *T. boudieri* led to the isolation of a new ketone and the known compound mannitol. The structures of the 2 isolated compounds were resolved using spectroscopic analysis including 1D and 2D Nuclear Magnetic Resonance (NMR), Infrared (IR) spectroscopy and were supported by literature data. The GC-MS analysis of the Fatty Acids (FAs) of *T. boudieri* oils showed high Unsaturated FA (UFA) profile (78.72%). The major FAs were linoleic acid (62.36%) and oleic acid (14.7%). Comparing the extracts obtained, the methanolic extract revealed the highest levels of Total Phenolic Content (TPC) of 185.56 mg GAE/100 g of truffle, thus the same extract showed the best results for antioxidant activity.

Keywords-*Terfezia boudieri*; methanolic extract; extensive spectroscopic analysis; fatty acids; GC-MS analysis; antioxidant activity

I. INTRODUCTION

Desert truffle is commonly used to describe ascomycete fungus organisms belonging mainly to two different genera: the genus *Terfezia* and the genus *Tirmania* of the family Terfeziaceae [1]. The fruiting bodies grow from mycorrhizal relationships with the host plants that inhabit sandy soils, slightly moistened by the rare rains of the desert or semi-desert region [2, 3]. In Arabian ecoregion, the desert truffle is socioeconomically important and is widely consumed and reputed [4]. According to the authors in [4-6], the local Bedouin population greatly appreciates the fruiting bodies of truffles for their delicious taste and also their nutritional value. The authors in [5] reported more than 20 fungal species belonging to the genus *Terfezia*. *T. boudieri* Chatin and *T. clavaryi* Chatin, which are reported to be spring species and relatively common during rainy years [6]. In the northern region of Saudi Arabia, as well as for many Nomads population, desert truffles are involved in the preparation of several dishes specific to the local Bedouin population. From North Africa to Middle East, desert truffles are known, among others, under different vernacular names: "Terfes", "Kama'ah", "Fagaa", "Azobaidi or white Kama'ah". The nutritional value and traditional health-benefits of desert truffles are well documented [7-14]. Desert truffles have been reported as a source of carbohydrates (65-67%), proteins (16.3-18.5%), lipids (6.2-5.9%), and amino acids [13]. Previous research reported the presence of mycochemical compounds such as sterols (ergosterol) and ceramides [15, 16]. Furthermore, desert truffles represented a promising source of nutraceuticals and fungochemicals with antioxidant, anti-inflammatory, and cytotoxic properties, as well as neurotrophic, and immune stimulant potentialities [15, 17-20]. Moreover, the bioactivity of desert truffles has attracted attention from the scientific community with ongoing interest in their exploitation and valorization. *Terfezia boudieri* is a native fungal species of North Saudi Arabia, is highly harvested by local nomadic populations as a food source and is known for its multiple medicinal properties. In the present investigation, we focus on the nutraceutical values (polyphenol content and Fatty acids composition) of *Terfezia boudieri* from Saudi Arabia. Fatty Acid (FA) profiling was carried out by analytical techniques through gas chromatography (GC) and GC/mass spectrometry (MS). Furthermore, extraction and purification of pure compounds were conducted, and their structure identification was contacted by Infra-Red (IR) and Nuclear Magnetic Resonance (NMR). Moreover, the antioxidant activity of fungal extracts was investigated via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for the studied fungal species.

II. MATERIAL AND METHODS

A. Desert Truffles: Fungal Material Collection and Identification

The fruiting bodies of desert truffles belonging to *T. boudieri* Chatin were harvested in the wild in February 2022. Fungal material was collected from different localities in Al Chathi tributary about 30 Km west of Arar City, Saudi Arabia (31° 11' 07'' N; 40° 50' 32'' E). The fungal material was examined and identified in the Department of Biology of the

Faculty of Sciences of the Northern Border University. The fruiting bodies were collected and stored at 4 °C for 2 hours prior to further analysis.

B. Preparation of Fungal Material Extracts

The dried truffle material from *T. boudieri* (250 g) was powdered and Soxhlet-extracted for 6 h at 45 °C. The fungal extracts were prepared by maceration in three different solvents (n-hexane, dichloromethane, and methanol). The extraction procedure was carried out as follows: 500 mL of the different macerates were filtered through a Whatman filter paper using anhydrous magnesium sulfate. In order to collect the fungal extract, the solvent was removed, at 40 °C, using a rotary evaporator (Rotavapor R-210, Büchi, Switzerland). The methanol extract was chromatographed over Silica gel column (60 × 6 cm, 70–230-mesh, i.d.), eluted with CH₂Cl₂, and a gradient of increasing polarity with methanol, leading to six main fractions (F1–F6). Fraction F4 (428 mg) was separated by flash column chromatography on silica gel (70–230-mesh), using CH₂Cl/MeOH (85:15) as eluents, yielding compound (1) (56 mg). The precipitation of fraction B-5 in methanol produced an impure solid, which was recrystallized using a small volume of methanol (2.5 mL) to obtain compound (2) (128 mg).

C. General Experimental Procedures

Thermo Scientific Nicolet iS5 Infrared Spectrometer was used for the Infrared (IR) spectroscopy characterization. The used method was the KBr pellet method. The range was fixed in the 400–4000 cm⁻¹ range with a resolution of 4 cm. A double-beam spectrophotometer (Jasco V-670) was used for the UV–Visible spectroscopy measurements, which is equipped with 1 cm wide quartz cells. The spectra were recorded at the 190 and 800 nm range.

D. Standards and Reagents

High grade solvents, purchased from Fisher Scientific UK, were used for extraction and analyses. High-purity grade Silica gel with a pore size 60 Å, 70-230 mesh 63-200 mm and TLC silica gel 60 F254, from Sigma Aldrich, were used for column chromatography. For the DPPH assay, analytical grade 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich.

E. Fatty Acid Composition

Oil samples representing n-hexane extract were used to prepare Fatty Acid Methyl Esters (FAMES) fractions. The FAMES were prepared according to a previously described protocol with slight modifications [21]. Briefly, FAMES were obtained using a mixture of sodium hydroxide dissolved in anhydrous methanol and added to the oil samples with continuous heating and stirring. At the end of the process, the top phase FAMES were separated, washed with distilled water, and dried with anhydrous sodium sulfate. For further analysis, 1 mL of the obtained FAME aliquots were injected into an analytical gas chromatograph instrument [22]. GC–MS chromatograph (QP2010 Ultra, Shimadzu, Japan), equipped with an FID detector and an Rxi-5Sil MS column (30 mm, 0.25 mm i.d., 0.25 mm film thickness), was used for FAMES separation. Helium (He), supplied at a flow rate of 1.50

mL/min, was used as the carrier gas. The oven temperature was arranged to reach temperatures varying from 150 °C to 180 °C with a rate of 15 °C/min and followed by an increase to 210 °C at 18 °C/min. For both injector and detector, the temperature was fixed at 220 °C.

F. Total Phenolics and Flavonoid Contents

The Folin-Ciocalteu method, as described by several authors [23, 24], was used for the determination of Total Phenolics Content (TPC). Briefly, the used protocol consists of 10 µL of sample extract (methanol, dichloromethane, n-hexane) mixed with distilled water (490 µL) and Folin-Ciocalteu reagent (500 µL). Saturated sodium carbonate (500 µL; 1%) was added, and for homogenization, the mixture was vortexed for 1 min. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer (JASCO), after an incubation period of 2 hours at room temperature. A standard curve of gallic acid was used for the calculation of the TPC. Moreover, for the calibration curve the used linearity range was 100–2000 mg/L) ($R^2 = 0.999$). Results were expressed and presented as the means of triplicate analyses i.e. milligrams of Gallic Acid Equivalents per g Dry Weight (mg GAE/gdw). Aliquots of 1 mL of fungal extract samples were mixed with 1 mL of the $AlCl_3$ solution (2% prepared in methanol). The optical density of the mixture was recorded after a 10-minute incubation period time at 430 nanometers. The standard sample was prepared using methanol instead of fungal extract. The calculation of Flavonoids Content (FC) was deduced on the basis of curve calibration established with quercetin (0.35 µg/mL). The results were presented in milligrams Quercetin Equivalents per 100 g of extract: mg QE/100g of extract [25].

G. DPPH Assay

According to the protocol described by several authors [26, 27], the used method is based on a relatively stable radical DPPH. Briefly, the DPPH solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. 50 µL of extract solution of *T. boudieri* and the used standard (ascorbic acid) were added to 1.95 mL of DPPH solution. The mixture was incubated for 30 minutes in darkness. The negative control contains only the DPPH solution. Optical density was measured at 517 nm using a UV/visible spectrophotometer. The percentage of radical-scavenging activity, RSA, was calculated with the following equation:

$$RSA(\%) = [(A_{517\text{control}} - A_{517\text{sample}})/A_{517\text{control}}] \times 100 \quad (1)$$

where $A_{517\text{control}}$ is the absorbance of the negative control reaction and $A_{517\text{sample}}$ is the absorbance of the fungal extract sample. The inhibitory concentration, IC_{50} , which signifies the antioxidant concentration that scavenges 50% of the initial DPPH radicals, was calculated from the graph plotting RSA to sample concentration. The lower the IC_{50} , the higher the antioxidant potential.

III. RESULTS AND DISCUSSION

A. Phenolic and Flavonoid Contents

The TPC of *T. boudieri* extracts was assessed by the Folin-Ciocalteu assay. As shown in Figure 1, the TPC in different extracts of the fungal species *T. boudieri* ranged between 11.2

and 185.4 mg GAE/gdw, presenting the highest value of 185.56 mg GAE/gdw in methanolic extract, followed by dichloromethane extract (126.3 mg GAE/gdw). The n-hexane extract exhibited the lowest TPC value with 11.2 mg GAE/gdw. The FC in the three studied extracts of *T. boudieri* was determined by the spectrophotometric method. The FC in the studied extracts ranged from 8.21 to 108.26 mg QE/100g of extract. The methanolic extract was found to have the highest FC (Figure 1). The dichloromethane extract exhibited an FC of 76.40 ± 0.63 QE/100g of extract and the FC in hexane was found at 8.21 ± 0.42 QE/100g of extract. Complementing these results with the literature, the TPC and FC in the *T. boudieri* truffle are slightly higher than other edible mushrooms [5, 27]. Furthermore, these results coincide with the work in [28], in which are presented a TPC of 159.67 ± 0.08 GAE/gdw) and a FC of 96.18 ± 0.52 a RE/gdw) for a methanolic extract of Tunisian *T. boudieri*.

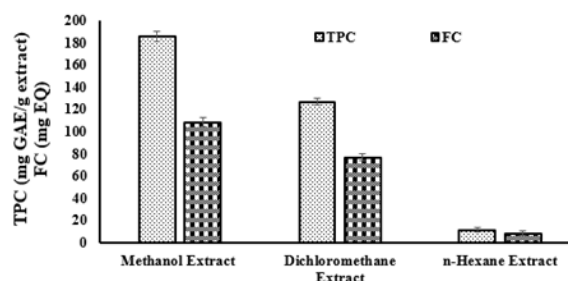


Fig. 1. TPC and TFC in fruiting bodies extracts of *T. boudieri* from northern region of Saudi Arabia.

B. DPPH Radical-Scavenging Activity

DPPH radical scavenging assay of the different fungal extracts was evaluated by the inhibitory potential of DPPH on a methanolic solution measured at 517 nm. Ascorbic acid was used as a standard. Figure 2 shows the antioxidant potential of the *T. boudieri* extract compared to ascorbic acid as a positive control.

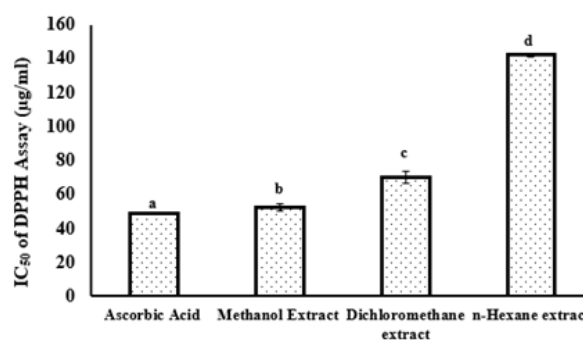


Fig. 2. IC_{50} of the DPPH assay (µg/mL) of fruiting bodies extracts of *T. boudieri* from the northern region of Saudi Arabia.

All desert truffle extractions (*T. boudieri*) have been found to show significant differences in radical scavenging activity. Moreover, methanol extract showed the strongest DPPH radical scavenging activity with an IC_{50} of 52.62 ± 0.26 µg/mL, followed by dichloromethane and n-Hexane extract

with IC₅₀ values of 70.34 ± 0.18 µg/mL and 142.52 ± 0.32 µg/mL, respectively. The positive control, ascorbic acid, showed an IC₅₀ value of 49.41 ± 0.38 µg/mL. The anti-radical activity of these extracts could be influenced by the presence of phenolic compounds in the extracts [29]. Furthermore, these results showed a good similarity to the results of several publications, which reported a positive correlation between TPC and antioxidant activity [19, 29-31].

C. Fatty Acid Composition of *T. Boudieri*

The total oil yield of the studied *T. boudieri* truffle (TBO) was 8.94%, which agrees with the results found for other truffle species from Tunisia, Algeria, and Iraq [32, 33]. As shown in Table I, the FA composition of TBO was expressed as a percentage of the total FA content. Moreover, eleven components, representing 99.92% of the total identified FA, were identified and quantified in the studied fungal oil. The studied oil was characterized by a high content of Total Unsaturated FAs (TUFA) of 78.7% and Total low Saturated FAs (TSFA) of 21.27%. Besides, the profiling showed a TUFA to TSFA ratio of 23.08%. Furthermore, the major FAs were linoleic acid (C18:2Δ9c, 12c) with a percentage of 62.36% and Oleic acid (C18:1Δ9c) with a percentage of 14.7%. Our results are in agreement with previous studies that presented the richness of truffles in FA [30,31]. Although the oil from *T. boudieri* grown in Northern region of Saudi Arabia had the highest amount of linoleic acid compared to the same desert truffle widely distributed in Northern Africa (54.10%), this variability in the chemical composition of oils could be due to many factors, such as environmental, climatic, nature of soil and geographic conditions [34-37].

TABLE I. FATTY ACID COMPOSITION (%) OF TBOS.

N°	Fatty acid	Name	Content (%)
1	C14:0	Myristic acid	0.2
2	C16:0	Palmitic acid	17.74
3	C16:1Δ9c	Palmitoleic acid	0.58
4	C18:0	Stearic acid	2.33
5	C18:1Δ9c	Oleic acid	14.7
6	C18:2Δ9c,12c	Linoleic acid	62.36
7	C20:0	Arachidic acid	0.17
8	C18:3Δ9c,12c,15c	Linolenic acid	0.73
9	C20:2Δ11c,14c	Eicosadienoic acid	0.35
10	C22:0	Behenic acid	0.72
11	C24:0	Lignoceric acid	0.11
	TSFA		21.27
	TUFA		78.72
	TUFA/T SFA		3.70

D. Structural Elucidation of Compounds 1 and 2

For the structural elucidation of the purified compounds, we used a combination of 1D, 2D NMR and high-resolution spectrometry, as well as the available literature data. The fungochemical analysis of the desert truffle *T. boudieri* collected in the Northern Region of the Saudi Arabia region is presented here for the first time and showed the presence of two compounds (1) and (2) as shown in Figure 3.

Compound (1) was isolated as a colorless oil and defined with the molecular formula (C₁₂H₂₀O₆). The compound was determined by HR-ESI-MS at m/z 283.11472 [M + Na]

(calculated for C₁₂H₂₀O₆Na: 283.11480 (Figure 3). [α]_D = +14.2 (H₂O, C 0.10); The IR bands of the compound indicated the presence of ketone and hydroxyl groups (1731 and 3650 cm⁻¹). As shown in Table II, the ¹H NMR spectrum showed the presence of four methylene protons at δH 3.805 (2H, dd, J = 11.2, 5.8 Hz), 2.764 (2H, m), 1.852 (2H, m), 3.572 (2H, dd, J = 11.8, 5.2 Hz), one methine hydrogen at δH 4.176 (H, t 6.8). The ¹³C and HSQC spectra showed that it contained four methylene carbons (δC 63.2, 34.7, 25.7 and 60.6), one methine (δC 77.8) and a ketone carbonyl carbon (δC 211.8). As explained above, the results showed only half the number of resonance signals expected for 20 protons and 12 carbons, the structure should be a symmetrical dimer, which supports that compound 1 is a dimeric derivative. HMBC correlations from H-1 to C-2 and C-3, from H-2 to C-3 from H-6 to C-4 and C-5 as well as the spin systems from H-1/H-2, H-4/H-5, and H-5/H-6 deduced from the 1H-1H COSY spectrum, revealed that C-1/C-2/C-3/C-4/C-5/C-6 form a -O-CH₂-CHO-CO-CH₂-CH₂-CH₂OH is a monomer moiety. Figure 4 shows the connection of the two dimers in C-1 to C1' and C-2 to C-2' through the oxygen atom and this was confirmed by the two correlations deduced from the HMBC spectrum (Figure 5).

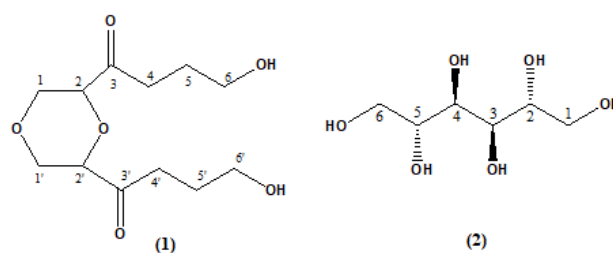


Fig. 3. Compound (1) and (2): isolated from fruiting bodies methanol extract of *T. boudieri* from northern region of Saudi Arabia.

TABLE II. ¹H AND ¹³C NMR DATA (500 AND 125 MHZ, CD₃OD) FOR COMPOUND (1).

	H [δ, mult, J (Hz)]	δC
1,1'	3.805, dd, 11.2, 5.8	63.2
2,2'	4.176, t, 6.8	77.8
3,3'	-	211.8
4,4'	2.764, m	34.7
5,5'	1.852, m	25.7

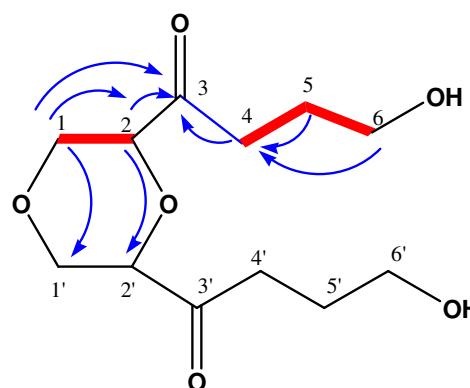


Fig. 4. Relevant COSY () and HMBC (H → C) correlations in compound (1).

Compound (2) was purified as a white amorphous powder [α]D = +14.2 (H₂O, C 0.10); HR-ESI-MS at m/z 205.06812 [M + Na] (calculated for C₆H₁₄O₆ Na: 205.24791; mp 152.5-153.0; ¹H-NMR (500 MHz, D₂O) δ : 3.80 (2H, dd, J = 11.0, 2.6 Hz, H-1a, 6a), 3.60 (2H, dd, J = 11.0, 5.8 Hz, H-1b, 6b), 3.69 (2H, m, H-2, 5), 3.72 (2H, d, J = 8.6 Hz, H-3, 4), ¹³C-NMR δ : 70.9 (C-2, 5), 69.1 (C-3, 4), 63.2 (C-1, 6).

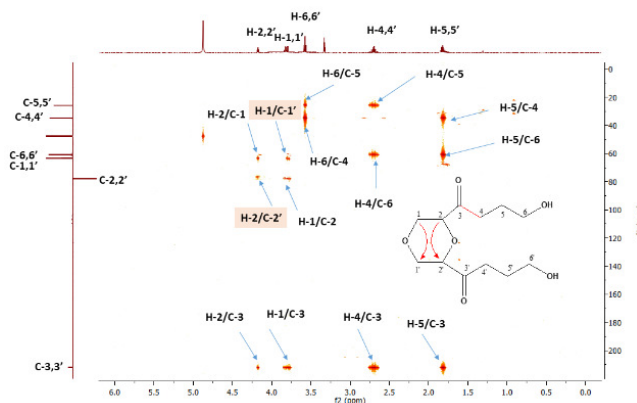


Fig. 5. HMBC spectrum of compound (1).

IV. CONCLUSIONS

Phytochemical investigation of *Terfezia boudieri* (*Terfeziaceae*) growing in Northern Region of Saudi Arabia. showed the isolation of a ketonic dimer named (1) and mannitol (2). The total oil yield for the studied fungal species was 8.94 %. The oil was characterized by the highest linoleic acid content (62.36%). In addition, the methanolic extract showed the highest value of phenolic and flavonoids compounds, exhibiting high antioxidant activity.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University for funding this work through the project number (SCIA-2022-11-1745).

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