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ANTIMICROBIAL AND SCAVENGING PROPERTIES OF *TERFEZIA BOUDIERI* CHATIN, A NATURAL EATABLE TRUFFLE FROM EGYPTIAN DESERT

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Abstract

Desert truffles of the genus *Terfezia* are valued for their nutritional richness and traditional medicinal use, yet scientific characterization of species growing in the Egyptian desert remains limited. *Terfezia boudieri*, in particular, is widely consumed but underexplored in terms of its bioactive potential. To address this gap, the present study aimed to evaluate the nutritional composition, chemical constituents, and biological activities of *T. boudieri*. The objectives included assessing its antioxidant capacity, determining its antimicrobial activity against pathogenic bacteria and fungi, and examining the structural effects of its extracts on selected microorganisms. Fresh truffle samples were analyzed for macronutrients, sugars, organic acids, phenolic acids, and fatty acids. Antioxidant activity was measured using scavenging assays, while antimicrobial effects of different extracts were evaluated by inhibition zone assays. Transmission and scanning electron microscopy were employed to observe ultrastructural alterations in the most affected microbes. The truffle showed notable nutritional value, with carbohydrates (15.3 g/100 g), proteins (9.03 g/100 g), ash (4.47 g/100 g), and fats (3.82 g/100 g) as major components. Arabinose (3.02 g/100 g), citric acid (5.04 g/100 g), and p-hydroxybenzoic acid (10.65 g/100 g) were the dominant sugar, organic acid, and phenolic acid, respectively. Palmitic, oleic, and linoleic acids were the primary fatty acids (44.8%, 19.04%, and 11.37%). Methanolic extract exhibited the strongest antioxidant effect (IC_{50} = 35.7 ± 0.3 µg/ml) and the highest antimicrobial activity against all tested bacteria and fungi. Microscopy revealed pronounced structural damage in *Aspergillus flavus*, *Candida glabrata*, *Salmonella typhi*, and *Staphylococcus aureus* following treatment. These findings highlight *T. boudieri*, particularly its methanolic extract, as a promising natural source of antioxidant and antimicrobial agents with potential therapeutic applications.

Key words: *Terfezia boudieri*; Antimicrobial; Antioxidant; Macronutrients; Electron microscope.

Introduction

Desert truffles of the genus *Terfezia* (family Pezizaceae) are edible hypogeous fungi that naturally occur in arid regions of the Middle East and North Africa, where they form mutualistic ectomycorrhizal relationships with *Helianthemum* species (Tang *et al.*, 2007; El Enshasy *et al.*, 2013; Al Obaydi *et al.*, 2020; Satish *et al.*, 2022). These fungi are valued not only for their distinctive flavor but also for their rich nutritional profile, which includes significant levels of proteins, carbohydrates, and bioactive metabolites (O'Donnell *et al.*, 1997; Kagan-Zur and Roth-Bejerano, 2008). Their ecological adaptability to low-oxygen, nutrient-poor desert environments is associated with the production of diverse secondary metabolites, many of which have been reported to possess antioxidant, antimicrobial, and other therapeutic properties (Murat *et al.*, 2004; Morte *et al.*, 2008).

Despite the traditional consumption and economic importance of *Terfezia* species, scientific data on *Terfezia boudieri*, especially those growing naturally in Egyptian deserts, remain limited **(**Honrubia *et al.*, 1992; Pacioni and Comandini, 1999; Fidan *et al.*, 2022**)**. Previous studies highlight truffles as a promising yet underexplored source of natural bioactive compounds, suggesting potential applications in modern medicine. However, comprehensive assessments integrating nutritional analysis, volatile profiling, and antimicrobial evaluation are still scarce for *T. boudieri* **(**Diez *et al.*, 2002; Bonito *et al.*, 2010; Aldebasi *et al.*, 2013; Sawaya *et al.*, 2023).

Therefore, the present study aims to characterize the nutritional composition and major chemical constituents of *T. boudieri* collected from Egyptian desert regions. Volatile components were analyzed using gas chromatography, while antioxidant potential was assessed through scavenging assays. Additionally, the antimicrobial activity of different extracts was evaluated against a range of bacterial and fungal pathogens, and microscopic examinations (SEM and TEM) were performed to elucidate the structural effects of the most active extracts on selected microorganisms. Together, these investigations provide an integrated evaluation of the potential therapeutic value of *T. boudieri* as a natural antimicrobial and antioxidant source.

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Materials and Methods

Truffle sample: Authenticated new ascocarps of *T. boudieri* (ARC0032) were obtained from Cairo University's Agriculture Research Centre, Cairo Egypt in March 2022.

Preparation of the extracts: There were two techniques used to extract *T. boudieri*'s bioactive molecules:

A: Aqueous extraction: The obtained *T. boudieri* fruits were washed, chopped into tiny pieces, and allowed to air dry at ambient temperature in a shaded area. Using a mechanical blender, the dried material was crushed into a fine powder and then run through 24 mesh sieves. At 37°C, 100 g of *T. boudieri* powder was processed by 50.0 mM sodium phosphate. To remove the majority of the debris, the extract was filtered using cheese cloth. The filtrate was then centrifuged for 20 minutes at 5°C at 15,000 rpm. For experimental purposes, the supernatant was left at 5°C and regarded as a crude aqueous extract of *T. boudieri* (Aldebasi *et al.*, 2012).

B: Solvent extraction: A 30 g sample of powdered fungus was mixed with 250.0 mL of chloroform or methanol or acetone in an 8-hour hotplate extraction at 54°C in a glass beaker. The final extract was concentrated at 41°C at low pressure in a rotary evaporator. Following the completion of extractions, all extracts were dried and kept at 5°C for storage (Doğan *et al.*, 2013).

Test microorganisms: All microbial strains used in this study were obtained from the Regional Centre of Mycology and Biotechnology, Al-Azhar University, Egypt. Bacterial isolates were maintained on nutrient agar slants, while fungal isolates were preserved on malt glucose agar slants, and all cultures were stored at 5°C.

***In vitro* assessment of antimicrobial properties of *T. boudieri* extracts':** Antimicrobial susceptibility testing was conducted using the well diffusion method. The diameter of inhibitory zones served as an expression for antimicrobial potentialities. The antibacterial properties of *T. boudieri* extracts were examined towards every type of microbiological isolate. All of the isolates of bacteria and fungus were applied as standardized inoculum suspensions (0.5 McFarland standard) onto the surface media. Using a sterile cork borer, four evenly spaced (1.0 cm size) holes were created in the plates (10.0 x 10.0 cm) that had been seeded with microbes for testing before being filled with 100 µL of each extract. As a negative control, discs were inserted with 100.0 µL of the corresponding 50.0 mM sodium phosphate buffer and solvents as negative controls. As positive references for bacteria and fungi, respectively, ciprofloxacin (100.0 µL /disc) and fluconazole (1.0 µL /disc) were utilized. After being chilled to (4.0 ±1.0°C) for an hour, the plates were kept for 24 hours at (35±3°C) for bacterial growth and 48 hours at (25±3ºC) for fungal growth. The inhibition zones for various extracts were measured three times, after being incubated for 24 to 72 hours (Doğan *et al.*, 2013). For the most affected microorganisms the MIC was determined by micro-dilution technique following a 24-hour incubation period at 37°C for bacteria and 3-5 days for fungi (Sehim *et al.*, 2023).

Nutritional potential: By applying the Anon., (2022) techniques, each specimen's chemical composition (proteins, fats, carbs, and ash) was examined. The macro-Kjeldahl method was used to estimate the amount of protein (N X 4.38); a Soxhlet device was used for separating an established weight of the specimen with petroleum ether to calculate the fat content; and burning at 600 ± 20ºC was used to assess the amount of ash. Carbohydrates were computed as follows: 100.0 - (g protein + g fat + g ash) = Carbohydrates. The formula used to determine energy was as follows: energy (kcal) = 4.0 X (g proteins + g carbs) + 9.0 X (g fat) (Carocho & Ferreira, 2013).

Chemical description of fruiting body hydrophilic chemicals

Free sugars: The 1 g of dried samples were extracted using 40.0 mL of 80.0% aqueous ethanol at 81°C for 90 minutes after being mixed by the Internal Standard, (raffinose, 5 mg/mL). The resultant suspension was defatted three times with 10 mL of ethyl ether each after being filtered and condensed by pressure using (Thermo R-310) rotary evaporator. The residues were concentrated, mixed in 5 mL of water, filtered through a disposable LC filter disc with a size of 0.22 µm, placed into an injection vial, and tested by a High-Performance Liquid Chromatography (HPLC) system. This system was comprised of an integrated pump (Agilent, system 2500), degassing step (Agilent, 2500), and auto-sampler (Agilent,2500), which were connected to a refraction index detection system. The Eurospher 100-5 NH2 column was utilized to accomplish the chromatographic separation.

(4.6 mm × 250.0 mm, 5.0 mm, Agilent) with a 7971R oven working at 35°C. Acetonitrile and deionized water were utilized as the mobile phase, 70.0:30.0 (v/v), with a rate of flow of 1.0 mL/min, and the volume of injection was 20.0 µL. By comparing the relative retention periods of sample peaks with standards, sugars were identified. Data Apex's Clarity 2.4 software was used to analyze the data. The findings were measured in grams for every 100 grams of dry weight (Weiß and Alt, 2017).

Organic acids: 1.5 g of lyophilized material were mixed with 25.0 mm of phosphoric acid at 25°C and 150 rpm for 45 minutes. The mixture was then filtered through Whatman No. 4 screen. Prior to HPLC (the apparatus previously stated) analysis, the Nylon 0.2 µm filters were used to filter the sample. A reverse phase C18 column (Thermo, 5.0 µm, 250.0 mm × 4.8 mm) subjected to 35°C was used for separation. A 0.81 mL/min rate of flow was used for the elution process utilizing 3.6 mM sulfuric acid. Using 220 nm and 245 nm as the preferred wavelengths, for detection (Wianowska and Olszowy-Tomczyk, 2023).

Phenolic acids' composition: 1.5 g of lyophilized materials were extracted over 6 hours at -20°C using methanol: water (80.0:20.0, v/v; 30 mL). Following a 15-minute sonication, the extract was passed through Whatman No. 4 paper and centrifuged at 4000 g for 10 minutes. Two further batches of the methanol: water combination, each 30 mL in volume, were used to extract

the residue. Methanol was extracted by evaporating the combined extracts at a lower pressure. After the aqueous phase was cleaned with hexane, diethyl ether (3.0 × 30.0 mL) and ethyl acetate (3.0 × 30.0 mL) were added to the liquid–liquid extraction. The organic phases were dried out, then re-dissolved in methanol (80:20, v/v; 1 mL) and filtered by 0.22 µm (Shahrzad & Bitsch, 1996; Wianowska & Olszowy-Tomczyk, 2023).

Fatty acids: After a transesterification process using the oil from the Soxhlet extraction, the fatty acids were methylated for at least 12.0 hours in a rotating bath at 50.0 ºC and 160 rpm using 5 ml of methanol, 2:1:1 (v:v:v) of H_2SO_4, and toluene. The next step was to add 3.0 mm of deionized water to accomplish phase separation. Fatty acid methyl esters, or FAME, were extracted using three milliliters of diethyl ether and vortex spinning. Anhydrous sodium sulphate was used to dry the upper phase. The specimen was retrieved in a vial with a Teflon barrier, and Millipore's 0.2 µlm nylon sift was used to purify it before injection. A gas chromatographer (DANI 1000) fitted with a flame ionization detector (GC-FID), a split/splitless injector, and a Thermo-Fisher column (0.26 l m df x m x 0.33 mm ID: 30) was used to carry out the analysis. The setting for the temperature of the oven was as follows: the column was first set to 50ºC, which was held for two minutes. After that, there was a 30-minute ramp to 125ºC, a 5-minute ramp to 160ºC, a 20-minute ramp to 180ºC, a 3-minute ramp to 200ºC, and a 20-minute ramp to 220ºC, all of which were held for fifteen minutes. At 50 κC, the carrier gas (hydrogen) flowrate was measured at 4.0 ml/min (0.61 bar). A split injection (1:40) with a volume of 1 µl was performed at 250 κC. Fatty acid identification was made by using CSW 1.7 software (Shah *et al.*, 2020).

DPPH radical scavenging action: A common method was used for the quantitative determination of the radical scavenging roles. 5 mm of 0.004% 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and fifty µL of the test sample (80% methanol as blank) were added to the reaction mixture. Ascorbic acid, a commercial antioxidant (Sigma), served as the study's reference. The optical density, evaluated at 520 nm, was recorded after 30 minutes of incubation (Sun *et al.*, 2020; Hsu *et al.*, 2021).

Transmission electron microscopy: After being exposed to *T. boudieri* extract, *Aspergillus flavus*, *Candida glabrata*, *Salmonella typhi* and *Staphylococcus aureus* cells were put for two hours with 2.6% glutaraldehyde. After fixation in 2.0% osmium tetroxide for two hours, the samples were stained with 1% uranyl acetate and dehydrated through a graded ethanol series. The resin was applied to embed the samples. An ultra-microtome (Leica, Germany) was used to segment the materials, and a transmission electron microscope (JOEL, Japan) was used to observe the sections (Sayed *et al.*, 2022).

Scanning electron microscopy: Preserved specimens of both normal and treated specimens by *T. boudieri* extract, including *Aspergillus flavus*, *Candida glabrata*, *Salmonella typhi* and *Staphylococcus aureus* cells, were examined on their exterior using scanning electron microscopy. Specimens were coated with gold, processed in an ethyl alcohol series, and subsequently seen under a scanning microscope (JOEL, Tokyo, Japan) (Sayed *et al.*, 2022).

Statistical analysis

Every experiment was in triplicates. Data were expressed as mean ± standard deviation (SD), and statistical analyses were performed using SPSS software.

Results and Discussion

In this study, extracts of *T. boudieri* exhibited inhibitory effects against all tested bacterial and fungal isolates could be seen in (Table 1). Gram positive bacteria exhibited the highest rate of sensitivity to *T. boudieri* methanol extract whereas the maximum inhibitory effect on *Staphylococcus aureus* in 3.6±0.2 cm to lowest inhibitory effect of aqueous extract on *Streptococcus pyogenes* of 0.4±0.1 cm, some extracts showed no inhibition zone towards some of the tested bacteria. Besides, Gram negative bacteria exhibited a promising rate of sensitivity to *T. boudieri* methanol extract towards *Salmonella typhi* in 2.1±0.2 cm to lowest inhibitory effect of aqueous extract on *Escherichia coli* of 0.4±0.2 cm. Furthermore, *T. boudieri* methanol extract showed a notable inhibition zone towards *Aspergillus flavus* of 2.3±0.2 cm, *T. boudieri* methanol extract showed the lowest inhibition zone towards *Trichophyton mentagrophytes* of 0.8±0.2 cm, some extracts showed no inhibition zone towards some of the tested fungal isolates. Lastly, *T. boudieri* methanol extract showed a highest inhibition zone towards *Candida glabrata* of 2.5±0.2 cm, *T. boudieri* chloroform extract showed the lowest inhibition zone towards *Trichosporon cutameum* of 0.3±0.2 cm, some extracts showed no inhibition zone towards some of the tested Yeast like fungal isolates. Collectively, methanol extract was the best extract exhibit antimicrobial activity followed by chloroform, acetone and aqueous extracts as compared with standard drugs (5 mg/disc) (Table 1).

Historically, the majority of research on truffles' pharmacological benefits has focused on their antibacterial properties found in desert truffles. For instance, *T. boudieri*'s antibacterial activity has been shown by agar-well diffusion experiments (Hamza *et al.*, 2016). There have been suggestions that these truffles could be useful in the management of dermatological and ocular conditions. Furthermore, several *T. claveryi* extracts have been shown to have antibacterial action versus *P. aeruginosa* (Gargano *et al.*, 2017).

This work has also shown that various *T. boudieri* extracts may contain distinct antibacterial components. Polar and nonpolar active ingredients are typically found in the methanolic extract, nonpolar chemicals predominate in the chloroform extract, and polar compounds are found in the aqueous extract. The specific processes underlying the antibacterial properties of the chemicals present in truffles have not been previously documented. Nevertheless, it has been suggested that terpenes, phenolic compounds, polysaccharides, anandamide may have an inhibitory

effect. Lectins have the ability to identify and remove bacterial exopolysaccharides (Passos da Silva *et al.,* 2019). Furthermore, it has been proposed that pathogenic bacteria are inhibited by superoxide anion radicals, which are released when phenolic compounds are oxidized and catalyzed by laccases (Nadim *et al*., 2015).

Phenolic compounds have strong anti-inflammatory, antibacterial, and antioxidant activities (Shinwari *et al.,* 2018; Anjum *et al.,* 2019; Jan *et al.,* 2022; Khan *et al.,* 2023). The phenolic compounds found in truffles may potentially have a role in their antibacterial properties. Numerous investigations have demonstrated the substantial activity of polyphenolic compounds against *B. subtilis*, and *E. coli* (Qin *et al.,* 2019). Thus, the antibacterial properties of the methanolic and ethyl acetate truffle extracts may be related to the actions of polyphenols such as *p*-hydroxy benzoic acid, rutin, gentisic acid, and cinnamic acid (Lima *et al.,* 2019). It's unclear how phenolic chemicals influence Gram-positive, Gram-negative bacteria, fungi and yeast differently in terms of their modes of action. The collective results of past research have shown that specific phenolic compounds as well as phenolic-rich extracts can suppress the growth of various harmful microorganisms (Metsämuuronen and Sirén, 2019; Khojah *et al.,* 2022). The minimal inhibitory levels of methanolic extract towards *Staphylococcus aureus, Salmonella typhi, Aspergillus flavus* and *Candida glabrata* were 62.5±1.20, 31.25±1.60, 250.0±3.60 and 125.0±1.20 µg/ml consequently (Table 2).

The results of the sugar profile, estimated energy value, and macronutrient composition of the dried *T. boudieri* powder are presented in (Table 3), showing defined levels of fats, proteins, carbohydrates, and total energy. The most prevalent macronutrient was protein (9.04 g/100 g), subsequent to carbohydrates (15.4 g/100g). There were minimal amounts of fat and ash (4.47 g/100g and 3.82 g/100g, consequently). The energetic level of *T. boudieri* specimen was 379.2 kJ/100g. In accordance with Reis *et al.,* (2012) who reported the nutritional impact of *Lentinus edodes* where ash content was (3.3%, dry weight). A protein, showed proximate levels (15.2%), fat content was (1.8–2.3%). Energetic level was (411 kcal/100g). In addition, Dejan *et al.,* (2013) who illustrated that the nutritional value of *Tirmani apinoyi* were estimated as carbohydrates (82.70 g/100g), proteins (8.07 g/100 g), Ash (5.26 g/100.0g) and fat levels (4.09 g/100 g). The energetic level of *T. pinoyi* specimen was 2450.20 kJ/100.0 g.

Table 1. *In vitro* antimicrobial action of *T. boudieri* extracts towards various bacterial and fungal isolates (Data are expressed as means ±SD).

Solvent / Test microorganisms	Methanol extract	Chloroform extract	Acetone extract	Aqueous extract	Standard drug
Gram positive bacteria					
Staphylococcus aureus ATCC25923	3.6 ± 0.2	1.2 ± 0.2	0.5 ± 0.2	1.0 ± 0.2	3.8 ± 0.1
Bacillus subtilis ATCC6633	2.6 ± 0.1	0.9 ± 0.1	0	0	2.8 ± 0.2
Streptococcus pyogenes ATCC12344	1.5 ± 0.1	0	0	0.4 ± 0.1	1.6 ± 0.4
Streptococcus pneumonia ATCC33400	2.2 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	0	2.8 ± 0.3
Entrococcus faecalis ATCC 29212	1.2 ± 0.2	1.0 ± 0.1	0.9 ± 0.2	0	2.4 ± 0.1
Gram negative bacteria					
Escherichia coli ATTC25922	0.4 ± 0.2	2.0 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	1.9 ± 0.1
Pseudomonas aeruginosa ATTC27853	0	2.3 ± 0.2	2.0 ± 0.2	0	2.6 ± 0.2
Klebsiella pneumonia ATTC13885	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	2.0 ± 0.2	2.8 ± 0.1
Proteus vulgaris ATTC13315	0	0.5 ± 0.2	0	0	2.7 ± 0.3
Salmonella typhi ATTC14028	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	0.9 ± 0.2	2.8 ± 0.2
Fungal isolates					
Aspergillus flavus ATCC 2014	2.3 ± 0.2	1.3 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	2.8 ± 0.2
Aspergillus niger ATCC 2024	1.5 ± 0.1	0	2.0 ± 0.1	2.0 ± 0.2	2.9 ± 0.2
Aspergillus fumigatus ATCC 2030	1.0 ± 0.1	2.4 ± 0.1	2.1 ± 0.2	0	2.6 ± 0.4
Fusarium cladosporium ATCC 2124	00	1.5 ± 0.2	0	1.7 ± 0.1	1.9 ± 0.1
Alternaria alternate ATCC 2151	00	00	1.2 ± 0.1	1.1 ± 0.2	2.1 ± 0.2
Syncephalastrum racemusam ATCC 2160	1.2 ± 0.2	1.7 ± 0.2	1.0 ± 0.2	1.5 ± 0.3	2.5 ± 0.2
Penicillium chrysogenum ATCC 2170	00	1.0 ± 0.2	0	0	2.0 ± 0.1
Microsporum canis ATCC 2190	1.0 ± 0.2	00	1.4 ± 0.2	0.8 ± 0.2	1.8 ± 0.2
Trichophyton mentagrophytes ATCC 2198	0.8 ± 0.2	1.0 ± 0.2	1.9 ± 0.2	1.4 ± 0.2	2.4 ± 0.1
Yeast like fungal isolates					
Candida albicans ATCC 1023	1.9 ± 0.1	1.0±0.2	0.5 ± 0.1	1.2 ± 0.2	2.4 ± 0.2
Candida krusei ATCC 1024	2.2 ± 0.1	0	0.7 ± 0.1	0	2.8 ± 0.2
Candida glabrata ATCC 1025	2.5 ± 0.2	0.9±0.2	0	0	3.1 ± 0.2
Cryptococcus humicola ATCC 1030	1.8 ± 0.2	00	0	0.9 ± 0.2	2.3 ± 0.2
Geotrichum candidum ATCC 1041	1.4 ± 0.2	0.8±0.2	0	0.9 ± 0.2	2.6 ± 0.2
Trichosporon cutameum ATCC 1044	0.4 ± 0.2	0.3±0.2	0	0.9 ± 0.2	2.1 ± 0.2

Table 2. Minimal Inhibitory concentrations of the most impacted microorganisms by methanol extract of *T. boudieri* (Data are expressed as means ±SD).

Microorganisms	MIC of methanol extract of *T. boudieri*	MIC of standard drug
Staphylococcus aureus	62.5 ± 1.20 µg/ml	5.0 ± 0.20 µg/ml
Salmonella typhi	31.25 ± 1.60 µg/ml	5.0 ± 1.20 µg/ml
Aspergillus flavus	250.0 ± 3.60 µg/ml	8.0 ± 1.20 µg/ml
Candida glabrata	125.0 ± 1.20 µg/ml	16.0 ± 1.20 µg/ml

Table 3. Macronutrients values and free sugars, organic acids and phenolic composition in dried powder formulations of *T. boudieri* (Data are expressed as means ±SD).

Nutritional value (g/100 g)	
Ash	4.47 ± 0.04
Proteins	9.04 ± 1.85
Fat	3.82 ± 0.03
Carbohydrates	15.42 ± 1.86
Energy (kcal/100 g)	379.24 ± 0.29
Free sugars' composition (g/100 g)	
Mannitol	1.2 ± 0.06
Rhamnose	2.5± 0.05
Trehalose	2.74 ± 0.70
Fructose	0.20 ± 0.01
Arabinose	3.02 ± 0.04
Organic acids content (g/100 g)	
Ascorbic acid (vitamin C)	0.40 ± 0.01
Oxalic acid	1.12 ± 0.00
Citric acid	5.03 ± 0.23
Fumaric acid	1.04 ± 0.06
Quinic acid	0.23 ± 0.15
Malic acid	0.03 ± 0.01
Phenolic compounds (µg/100 g)	
p-Hydroxybenzoic acid	10.65 ± 9.92
Cinnamic acid	8.30 ± 0.55
Vanillic acid	0.08

Table 4. The Composition of each fatty acid in *T. boudieri*'s dried powder preparations (Data are expressed as means ±SD).

Fatty acids	Content	Fatty acids	Content (%)
C6:0	1.29 ± 0.01	C18:2n6c	44.9 ± 0.53
C8:0	0.33 ± 0.01	C18:3n3	4.22 ± 0.02
C10:0	0.05 ± 0.02	C20:0	0.22 ± 0.01
C12:0	0.08 ± 0.01	C20:1c	0.11 ± 0.00
C13:0	0.01 ± 0.01	C20:2c	0.16 ± 0.00
C14:0	0.49 ± 0.01	C20:3n3 + C21:	0.55 ± 0.03
C14:1	1.19± 0.04	C20:5n3	0.13 ± 0.01
C15:0	0.49 ± 0.02	C22:0	3.43 ± 0.07
C15:1c	0.51 ± 0.00	C22:1n9	0.24 ± 0.01
C16:0	11.38 ± 0.08	C22:4n6	1.26 ± 0.06
C16:1	0.10 ± 0.01	C23:0	1.51 ± 0.02
C17:0	0.76 ± 0.02	C24:0	1.76 ± 0.03
C17:1c	0.01 ± 0.00	SF	27.6 ± 0.29
C18:0	5.80 ± 0.10	MUF	21.21 ± 0.26
C18:1n9c	19.05 ± 0.27	PUF	51.04 ± 0.55

C6:0 (Hexanoic acid); C8:0 (Octatonic acid); C10:0 (Decanoic acid); C12:0 (Dodecanoic acid); C13:0 (Tricyclic acid); C14:0 (tetradecanoic acid); C14:1 (9-tetradecanoic acid); C15:0 (Pentadecylic acid acid); C15:1c (Pentadec-10-enoic acid); C16:0 (Hexadecanoic acid); C16:1 (Cis-Palmitoleic acid); C17:0 (Margaric acid); C17:1c (Isooleicid); C18:0 (Stearophanic acid); C18:1n9c (9-Octadecenoic Acid); C18:2n6c (cis-9,12-Octadecadienoic acid); C18:3n3 (Alpha-Linolenic acid); C20:0 (icosanoic acid); C20:1c (gondoic acid); C20:2c (eicosa-11,14-dienoic acid); C20:3n3 + C21:0 (eicosatrienoic acid and eicosa-11Z, 14Z, 17Z-trienoic acid); C20:5n3 (cis-5, 8, 11, 14, 17-Eicosapentaenoic acid methyl ester); C22:0 (1-Docosanoic acid); C22:1n9 (cis-13-docosenoic acid); C22:4n6: (cis-7, 10, 13, 16-Docosatetraenoic acid)); C23:0 (Tricosylic Acid); C24:0 (Tetracosanoic acid); C24:1 (Selacholeic acid). SF—saturated fatty acids; MUF—monounsaturated fatty acid; PUF—polyunsaturated fatty acids

T. boudieri included the following free sugars in order of concentration: rhamnose (2.5 g/100 g), mannitol (1.2 g/100 g), fructose (0.2 g/100 g), and arabinose (3.02 g/100 g) and trehalose (2.74 g/100 g) (Table 3). In addition to its roles as a transport sugar and store carbohydrate, trehalose also serves a variety of biological purposes. As an essential metabolite, it can shield plants, animals, and microbes from adverse environmental conditions like starvation, cold, and dehydration (Streeter, 2003). Trehalose (1.93 g/100g) and rhamnose (1.72 g/100g) were the two main free sugars detected in *Tirmania pinoyi*, with mannitol (0.31 g/100g) coming in next (Dejan *et al.*, 2013) as well as, mannitol and trehalose were the main free sugars present in *Lentinus edodes* sample (Carocho and Ferreira, 2013).

The findings for organic acids in *T. boudieri* are currently shown in (Table 3). Citric acid (5.03 g/100g) was the most abundant organic acid. Quantification of fumaric acid (1.04 g/100g) and oxalic acid (1.12 g/100g) was also achievable. While fumaric acid has intriguing biological effects like anti-inflammatory, protective of neurons, and chemo-preventive properties, citric acid is a crystal thickening agent in bones (Hu *et al.*, 2010). Both acids are vital for human metabolism and play a significant role in the Krebs cycle. Additionally, it has antibacterial properties that help preserve fruits and vegetables (Baati *et al.*, 2011).

Analysis of phenolic compounds revealed the presence of cinnamic acid, p-hydroxybenzoic acid, p-coumaric acid, and vanillic acid, with p-hydroxybenzoic acid being the predominant component (10.6 mg/100 g) and cinnamic acid also detected at notable levels (8.3 mg/100 g) (Table 3). These phenolic acids, belonging to the hydroxycinnamic and hydroxybenzoic groups, are important antioxidants known for their free-radical-scavenging and metal-chelating properties (Carocho & Ferreira, 2013).

The findings regarding *T. boudieri*'s fatty acid profile are shown in (Table 4). Following saturated fatty acids (SFA, 27.6% of total FA) and monounsaturated fatty acids (MUFA, 21.21% of total FA), polyunsaturated fatty acids (PUFA, 51.04% of total FA) were the most prevalent. In the specimen, oleic, palmitic, and linoleic acids constituted 44.9%, 19.05%, and 11.38% of the total FA, consequently. Longvah and Deosthale (1998) also noted the presence of PUFA in the fruiting bodies of L. edodes, primarily as a result of the contribution of linoleic acid (linoleic acid amounts: 69% and 81%). According to Rubio-Rodríguez *et al.* (2010), PUFA may offer protection from a number of diseases, including mental, rheumatologic, and cardiovascular conditions.

The scavenging impact of *T. boudieri* extracts evaluated by DPPH *in vitro* assay. Aqueous extract had highest IC_{50} = 221.9±0.2 µg/ml) followed by chloroform IC_{50} = 142.4±0.6 µg/ml), acetone IC_{50} = 91.6 µg) then methanol (IC_{50} = 35.7±0.3 µg/ml) extracts as compared with ascorbic acids (11.2±0.4 µg/ml) as illustrated in (Fig. 1). These outcomes demonstrated that the extraction solvents had a dramatic role on the DPPH activity. **(**Kıvrak, 2014) showed that *T. claveryi* from Turkey ethyl acetate extract showed the DPPH in with an IC_{50} of 57.73 µg/mL.

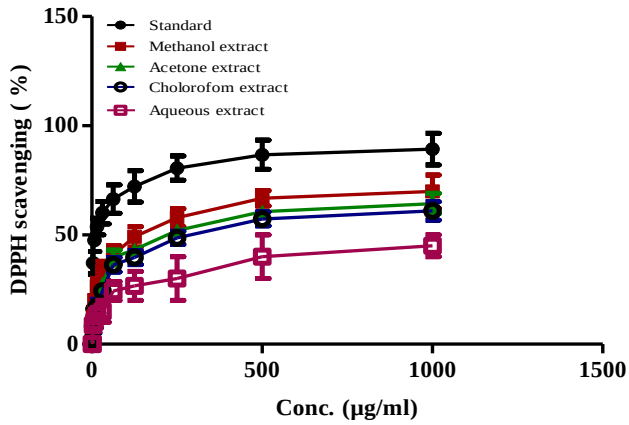

Fig. 1. Antioxidant impact of using DPPH scavenging activity of various extracts of *T. boudieri* versus ascorbic acid as a standard (Data are expressed as means ±SD).

The study aimed not only to document the nutritional and chemical composition of *T. boudieri* but also to determine whether its bioactive constituents contribute to antimicrobial activity. While inhibition assays demonstrate antimicrobial potential, they do not reveal how the truffle extracts affect microbial structures. Therefore, electron microscopy was used to visualize ultrastructural damage in the most affected bacteria and fungi, providing mechanistic evidence that links the truffle's chemical composition—particularly its phenolic and fatty acids—to its observed antimicrobial action. *Staphylococcus aureus, Salmonella typhi, Aspergillus flavus* and *Candida glabrata* were treated by various relevant MICs and the variations in the cellular structures were examined using both transmission and scanning electron microscopes. *Staphylococcus aureus* and *Salmonella typhi* could be seen in a rod classical form with regular internal organelles upon examination using transmission electron microscope, while upon treatment using *T. boudieri* methanol extract a disintegration of the internal structures of the cells, while upon examination using scanning electron microscope a shrinkage of the cellular volume could be seen (Figs. 2, 3). *Aspergillus flavus* with typical hyphal cell wall and cell membrane which distorted upon treatment using *T. boudieri* methanol extract and upon examination using transmission electron microscope, while upon examination using scanning electron microscope with typical conidiophore and conidia, while upon treatment using *T. boudieri* methanol extract a clear shrinkage in conidiophore and conidia could be seen (Fig. 4). Lastly, examination of *Candida glabrata* normal cells using transmission electron microscope revealed a well-organized cell wall and cell membrane as well as clear internal organelles, on the other hand treatment using *T. boudieri* methanol extract led to various holes in the cellular surfaces and degradation of the internal organelles, while upon using scanning electron microscope an aggregated rounded cells could be seen, on the other hand treatment using *T. boudieri* methanol extract led to separation of cell aggregates and disorganized cells could be seen (Fig. 5).

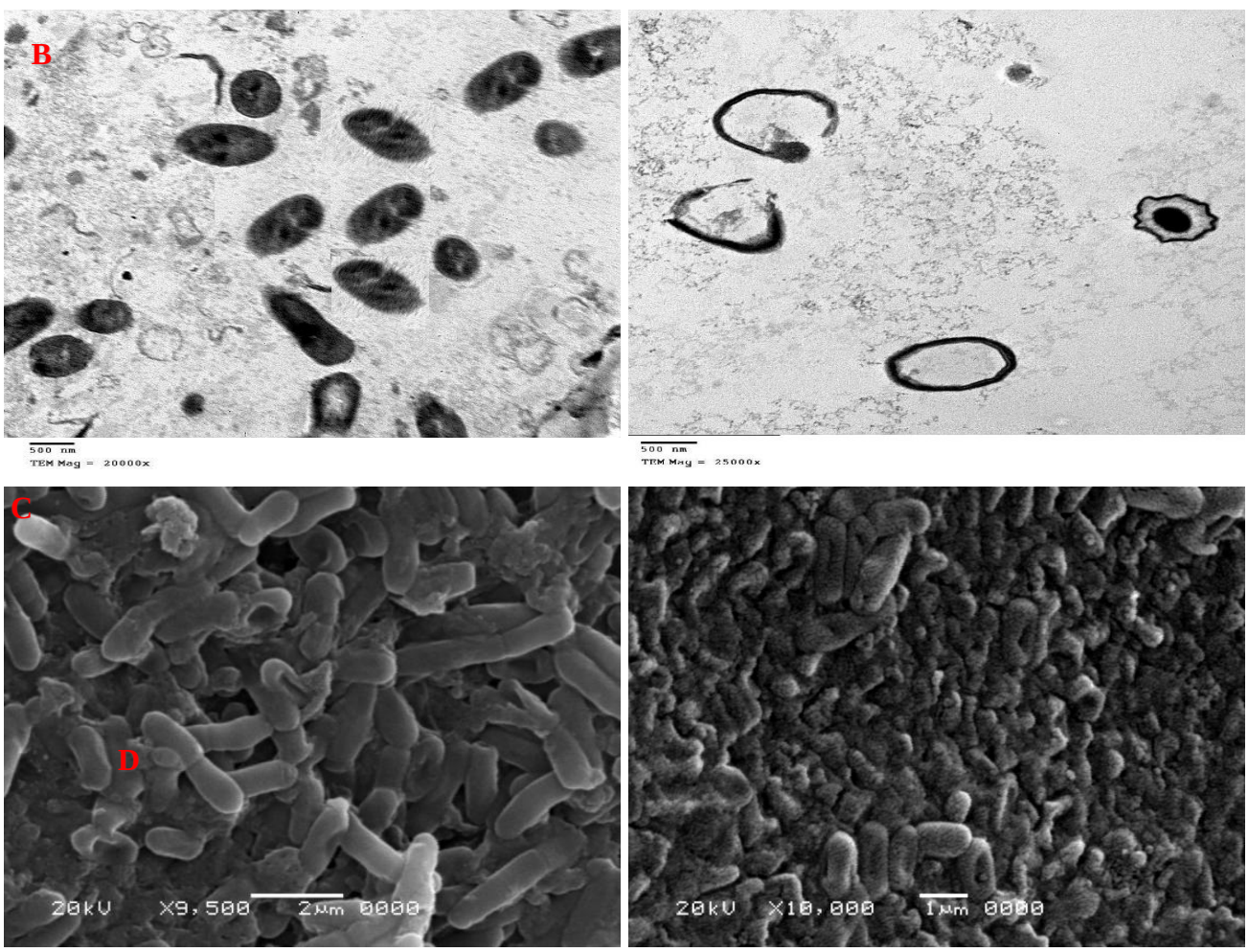

Fig. 2. Electron microscopic examination of normal *Staphylococcus aureus* and after treatment using MIC of methanol extract of *T. boudieri;* (A) Normal, (B) treated using transmission electron microscope; (C), Normal and (D) treated using scanning electron microscope.

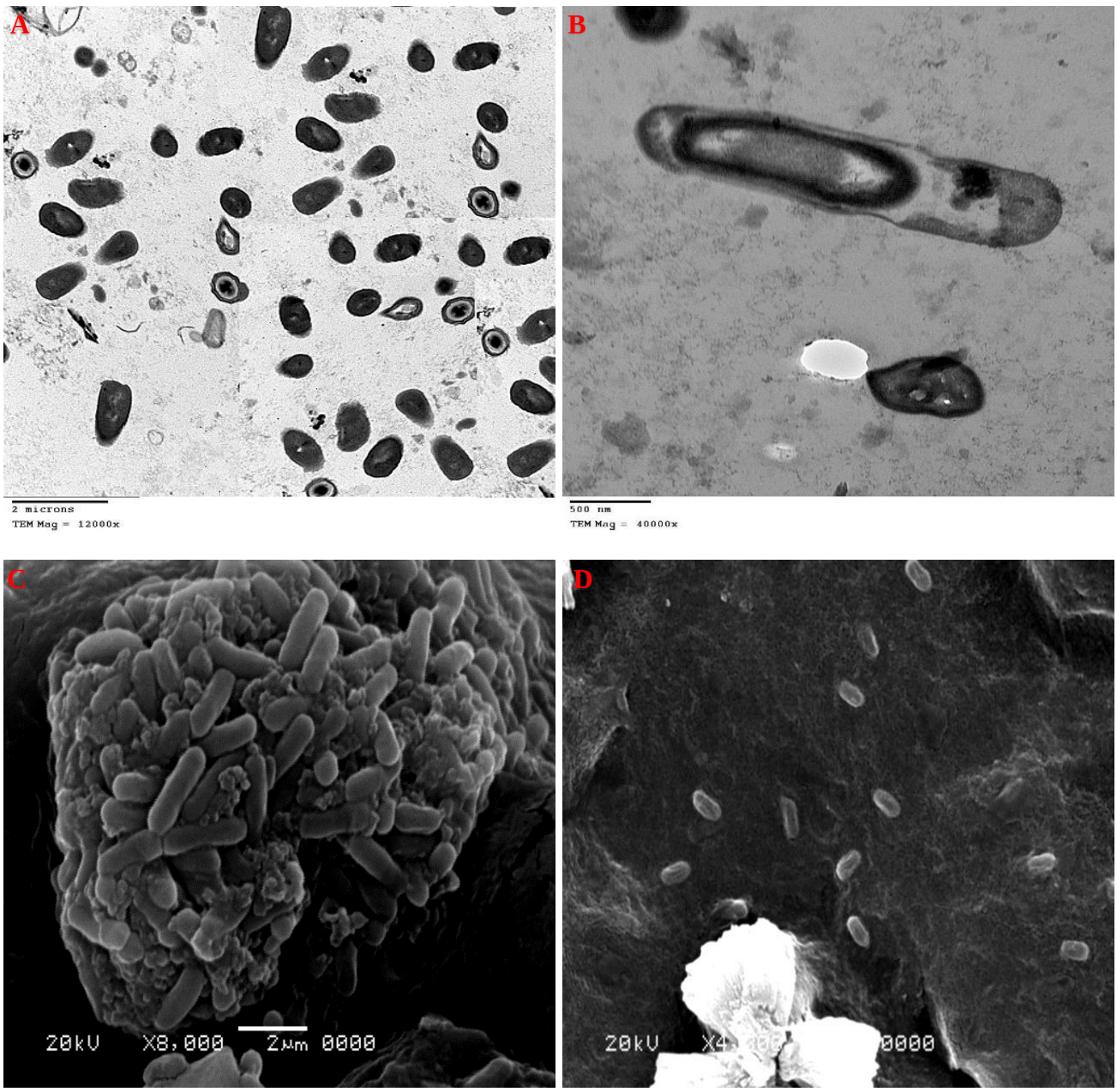

Fig. 3. Electron microscopic examination of normal *Salmonella typhi* and after treatment using MIC of methanol extract of *T. boudieri;* (A) Normal, (B) treated using transmission electron microscope; (C), Normal and (D) treated using scanning electron microscope.

Microscopic examinations are used to evaluate changes in examined microbial cells. It seemed that the molecules existing in the extracts had the ability to get through the peptidoglycan layer of the bacterial cell to the cell membrane and produce the antibacterial implications as well as alteration of cell membrane of fungi to produce antifungal impact. (Yang *et al*., 2017; Famuyide *et al.*, 2020)**.** The results of this work might offer additional suggestions into innovative alternate methods to control microbes by compromising the viability of the pathogen. To determine the antibacterial qualities as well as protective features of the bioactive components from the *T. boudieri* extracts from this study, it could be beneficial to separate and describe them which could be useful as modern antimicrobial agents.

Nutrition has a substantial impact on antimicrobial capacity, both in hosts (malnourished persons have lower defenses) and in natural products (more nutrition often indicate more beneficial chemicals like phenolics, which improve antibacterial capability). Malnutrition decreases the body's antimicrobial proteins, increasing the risk of infection, but excellent nutrition strengthens these defenses; conversely, natural product extracts high in nutrients and phytochemicals (phenolics, flavonoids) have better antibacterial properties (Shao *et al.*, 2021). Many substances that are natural, such as garlic, honey, and a variety of herbs and spices, have both major nutritional value and effective antibacterial capabilities, and there is a huge need to discover new substances due to the development of antimicrobial resistance (Stan *et al.*, 2021). The present results suggest the possibility of using *T. boudieri* collected from Egyptian desert due to high nutritional value and medicinal impact.

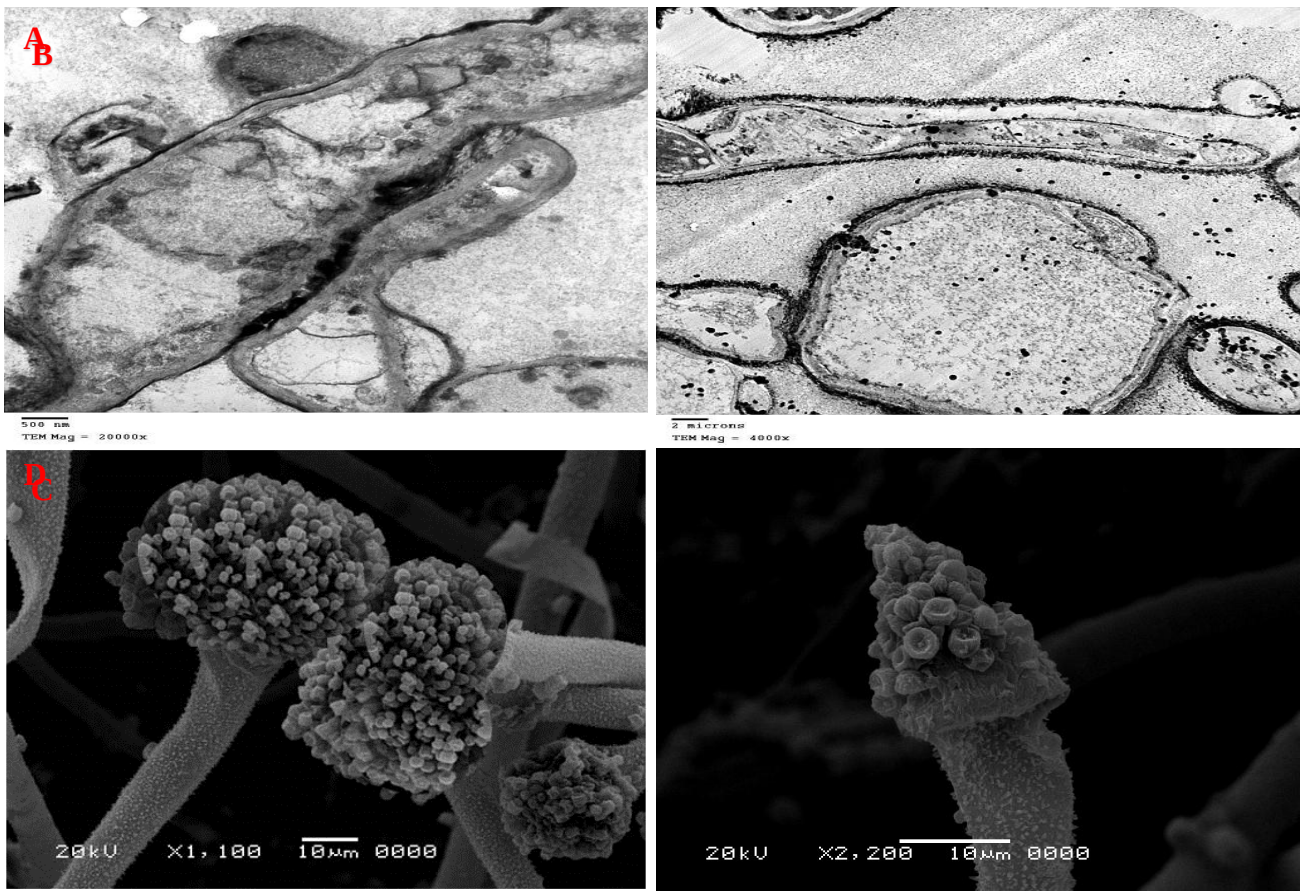

Fig. 4. Electron microscopic examination of normal *Aspergillus flavus* and after treatment using MIC of methanol extract of *T. boudieri;* (A) Normal, (B) treated using transmission electron microscope; (C), Normal and (D) treated using scanning electron microscope.

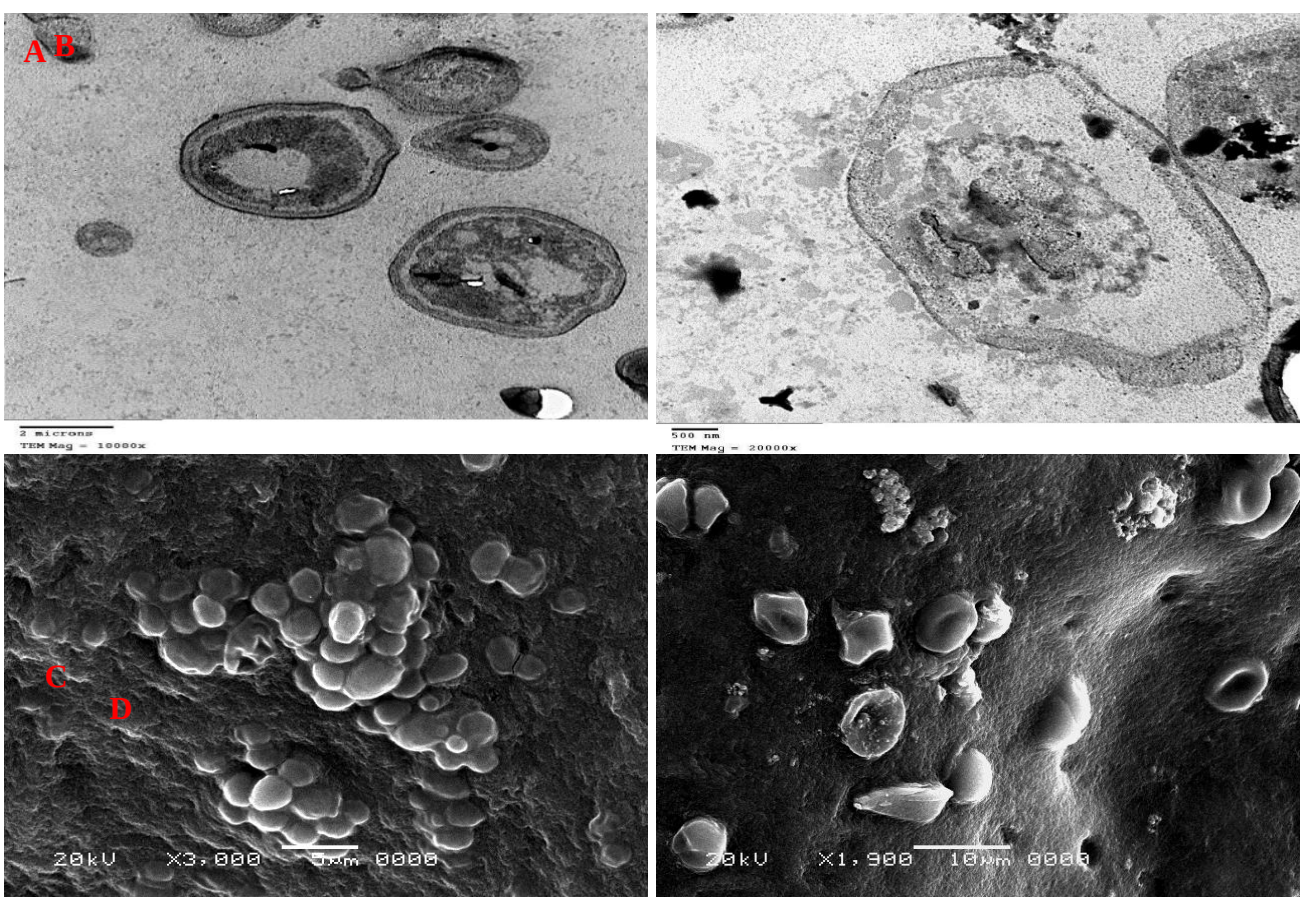

Fig. 5. Electron microscopic examination of normal *Candida glabrata* and after treatment using MIC of methanol extract of *T. boudieri;* (A) Normal, (B) treated using transmission electron microscope; (C), Normal and (D) treated using scanning electron microscope.

Conclusions

The current findings suggest that edible desert truffles in Egypt, which are important for the economy and have a pleasant taste, may also have noteworthy antioxidant and antimicrobial properties. Additionally, they are an excellent supply of fatty acids and contain substantial quantities of carbohydrates and protein, making them extremely intriguing from a nutritional standpoint. Future research in this field ought to focus on other edible and commercially significant desert truffles and mushrooms.

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