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RESEARCH ARTICLE

Mineral contents, Antioxidant and Antimicrobial Activities of Algerian *Terfezia claveryi* extracts

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SUMMARY

Terfezia species are known for their high nutritional value and diverse biological activities due to their unique chemical composition. The current study aims to evaluate the different extracts of Terfezia claveryi Chatin for their in vitro antioxidant, antibacterial, total phenolic content, total flavonoid content and mineral content. The antioxidant activity was evaluated via 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS and phosphomolybdenum assays. While the in vitro antibacterial activity was evaluated via disc diffusion method against Staphylococcus aureusas a Gram-positive bacteria as well as Escherichia coli and Pseudomonas aeruginosa as Gram-negative bacteria. The total phenolic content (TPC) and total flavonoid content (TFC) were evaluated via Folin-Ciocalteu's and $AlCl_3$ assays, respectively. The mineral content was evaluated via using atomic absorption spectrophotometer. In the antioxidant assays, the aqueous extract showed the most potent activity $IC_{50/DPPH}$ equal to 0.09mg/mL, $IC_{50/ABTS}$ equal to 0.24mg/mL and total antioxidant capacity equal to 0.10. Among all the tested extracts, the aqueous extract showed the highest TPC (176.67 mg GAE / g DW) and the highest TFC (14.53 mg ER / g DW). Obviously, the antioxidant activities are exist in a positive correlation with the TPC and TFC. On the other side, the tested extracts exhibited strong to moderate antibacterial activity against tested pathogenic microbial strains. The methanolic and aqueous extracts showed the most potent antibacterial activity against Pseudomonas aeruginosa with inhibition zones 21.91 and 15.79 mm, respectively. The extracts contain macroelements like Na (3293.01 mg· kg^{-1}dw) and K (21092.19 mg· kg^{-1}dw). Additionally, they contain micro-elements like Fe (16.45 mg· kg^{-1}dw), Cu (22.80 mg· kg^{-1}dw) and Ni (27.88 mg· kg^{-1}dw). LC/ESI-MS-MS was used to determine the chemical profile of the extract obtained from Terfezia claveryi. A total of seven phenolic compounds were selected. Rutin and hesperidin were primary compounds in the extract with 38.0089 and 22.4629 mg/g. In conclusion, T. claveryi extracts are considered promising sources of naturally occurring antioxidant and antimicrobial agents as well as their high nutritional values

Key Words: *Terfezia claveryi, Antioxidant, Antimicrobial, Mineral content, TPC, TFC*

Cezayir Terfezia claveryi Ekstrelerinin Mineral İçerikleri, Antioksidan ve Antimikrobiyal Aktiviteleri

ÖZ

Terfezia türleri, benzersiz kimyasal bileşimleri nedeniyle yüksek besin değeri ve çeşitli biyolojik aktiviteleriyle bilinmektedir. Bu çalışma, Terfezia claveryi Chatin'in farklı ekstrelerinin in vitro antioksidan, antibakteriyel, toplam fenolik içerik, toplam flavonoit içeriği ve mineral içeriği açısından değerlendirilmesini amaçlamaktadır. Antioksidan aktivite 1,1-difenil-2-pikrilhidrazil (DPPH), ABTS ve fosfomolibden analizleri yoluyla değerlendirildi. İn vitro antibakteriyel aktivite, Gram-pozitif bakterilerden Staphylococcus aureus'un yanı sıra Gram-negatif bakterilerden Escherichia coli ve Pseudomonas aeruginosa'ya karşı disk difüzyon yöntemiyle değerlendirildi. Toplam fenolik içerik (TPC) ve toplam flavonoit içeriği (TFC), sırasıyla Folin-Ciocalteu ve $AlCl_3$ analizleri ile değerlendirildi. Mineral içeriği atomik absorpsiyon spektrofotometresi kullanılarak değerlendirildi. Antioksidan analizlerinde sulu ekstre, DPPH radikaline karşı 0,09 mg/mL'ye eşit IC_{50}, ABTS radikaline karşı 0,24 mg/mL'ye eşit IC_{50} ve 0,10'a eşit toplam antioksidan kapasiteyi gösterdi. Test edilen tüm ekstreler arasında sulu ekstre en yüksek TPC'yi (176,67 mg GAE/g DW) ve en yüksek TFC'yi (14,53 mg ER/g DW) gösterdi. Antioksidan aktiviteler TPC ve TFC ile pozitif bir korelasyon içinde bulundu. Öte yandan, test edilen ekstreler, test edilen patojenik mikrobiyal türlere karşı güçlü ila orta dereceli antibakteriyel aktivite sergiledi. Metanolik ve sulu ekstreler, sırasıyla 21.91 ve 15.79 mm'lik inhibisyon bölgeleriyle Pseudomonas aeruginosa'ya karşı en güçlü antibakteriyel aktiviteyi gösterdi. Ekstraktlar Na (3293.01 mg.kg-1dw) ve K (21092.19 mg· kg^{-1}dw) gibi makro elementler içermiştir. Ek olarak Fe (16,45 mg· kg^{-1}dw), Cu (22,80 mg· kg^{-1}dw) ve Ni (27,88 mgkg^{-1}dw) gibi mikro elementler içerirler. Terfezia claveryi'den elde edilen ekstraktın kimyasal profilini belirlemek için LC/ESI-MS-MS kullanıldı.Toplamda yedi fenolik bileşik belirlendi.Rutin ve hesperidin, 38.0089 ve 22.4629 mg/g ile ekstrakttaki ana bileşikti. Sonuç olarak, T. claveryi ekstraktlarının yüksek besin değerlerinin yanı sıra doğal olarak oluşan antioksidan ve antimikrobiyal ajanlar açısından da umut verici kaynaklar olduğu düşünülmektedir.

Anahtar Kelimeler: *Terfezia claveryi, Antioksidan, Antimikrobiyal, Mineral içeriği, TPC, TFC*

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INTRODUCTION

Desert truffles are edible fungi with important gastronomic, nutritional and medicinal properties, locally named « terfess ». They are mostly endemic to semi-arid and arid areas of Arabian Peninsula, the North-Africa and Mediterranean Region (Kagan-Zur and Roth Bejerano, 2008). Desert truffles grow in arid desert climate characterized by very hot summers with high humidity and relatively cooler winters (Mandeel and Al-Laith, 2007). They are socio-economically important fungi and are widely consumed in North Africa (Morocco, Algeria, Tunisia and Egypt) and in the Middle East (Saudi Arabia, Kuwait, Iraq, Iran, Lebanon, Syria and Jordon), the productive area of desert truffles are characterized by its aridity and high average temperatures. At Northern Sahara of Algeria, three species of desert truffles have been identified: *Tirmania nivea* (Desf.) (Trappe 1971) , *Terfezia arenaria* (Moris, 1829) Trappe 1971 and *Terfezia claveryi* Chatin 1892 (Bradai et al., 2014).

The main nutritional constituents of truffle are carbohydrates, followed by proteins (Dahham et al., 2018, Khalifa et al., 2019; Lee et al., 2020). Most of their carbohydrates are considered dietary fibers such as chitin, -glucans and other polysaccharides, and they also include mannitol and trehalose (Tejedor-Calvo et al., 2020) as well as smaller sugars such as D-glucose, D-mannose or D-galactose (Tejedor-Calvo et al., 2020). Although truffles show low fat levels, their lipid content is important since they are involved in flavor and aroma properties. To maintain their hyphal membranes it is necessary to obtain unsaponifiable molecules such as ergosterol (ergosta-5,7,22-trienol), ergosta7,22-dienol, stigmasterol or ergosta-5,8-dieno-3-ol (Harki et al., 1996;Tang et al., 2012). Brassicasterol (ergosta-5,22-dienol) is also frequently detected in truffles; however, it is mainly reported in plants and algae species but is also found in species belonging to the subphylum Taphrinomycotina, a dimorphic plant parasite (Weete et al., 2012). Recently, truffles have shown interesting bioactive compounds, and their potential bioactivities are now being studied, e.g., antitumoral, antioxidant, immunomodulatory and hypoglucemic properties (Tejedor-Calvo et al., 2020; Bhotmange et al., 2019; Mudliyar et al., 2019; Farzaneh et al., 2018; Zhao et al., 2014). Therefore, the aim of this study is to evaluate the antioxidant, antibacterial activities and mineral analysis of different extracts of *T. claveryi*. Also, the phytochemical investigation was performed using LC-MS/MS.

MATERIALS AND METHODS

Sample extraction

T. claveryi truffles were collected from the Algerian northern Sahara region, cleaned, peeled, and sliced. The samples were dried in an oven at (35-40 °C) and ground mechanically. Approximately 10 g of the sample was extracted separately with 100 mL of different solvents (water, ethyl acetate, ethanol, methanol, acetone and chloroform) using the maceration method. The extracts were filtered and concentrated using a rotary evaporator (Buchi, USA) at 40 °C. Extracts were stored at -20 °C for further analysis.

Total phenolic content (TPC)

The concentration of total phenolic compounds was measured according to Bakchiche et al., 2022 with some modifications. A 0.1 mL sample of extract was mixed with0.2 mL of Folin-Ciocalteu's phenol reagent and 2 mL of water. The mixture was shaken and allowed to stand for 3 min, before addition of 1 mL of Na_2CO_3 (20 %). After the addition, the solution was incubated in the dark at room temperature for 30 min. Finally, the absorbance of the solution was measured at 765 nm and compared to a gallic acid calibration curve.

Total flavonoid content (TFC)

Total flavonoids were assessed using the method reported by Sun et al. with some modifications. A 1 mL sample of extract was mixed with 1 mL of 2% methanolic $AlCl_3$. The solution was incubated at room temperature for 10 min. Finally, the absorbance of the solution was measured at 430 nm.

Assay of DPPH scavenging activity

The DPPH radical scavenging activity was determined by a spectrophotometric method based on the reduction of methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). 200 µL of each extract at different solvents was added to 1.8 mL methanolic solution of DPPH (0.2 mM). The mixture was allowed to react at room temperature in the dark for 30 min. After 30 min, the absorbance (A) was measured at 517 nm. The experiment was repeated for three times for each test sample (Apriliyanti et al., 2020). IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. DPPH free radical-scavenging activity was calculated according to the following equation: DPPH radical-scavenging activity (%) = $(Abs_{control} - Abs_{sample}) / (Abs_{control}) \times 100$.Where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract / standard.

ABTS cation radical scavenging assay

ABTS radical scavenging activity was determined according to the method by Bakchiche et al., 2022 The $ABTS^+$ solution was prepared and stored in the dark at room temperature for 16 h. Then, 1 mL of the solution was diluted with 40 mL deionized water to yield working $ABTS^+$ solution with an absorbance equal to 0.70 ± 0.02 at 734 nm. To 1.485 mL ABTS+ working solution, 15 µL test samples were added. After 10 min, the absorbance of the plate was read at 734 nm.

ABTS free radical-scavenging activity was calculated according to the following equation: ABTS radical-scavenging activity (%) = (Abs control–Abs sample) / (Abs control) × 100. Where Abs control is the absorbance of ABTS radical + methanol; Abs sample is the absorbance of ABTS radical + sample extract / standard. The lower value of IC50 has the most critical antioxidant activity.

The extract concentration providing 50% radical scavenging activity (IC50) was calculated from the graph of ABTS·+ scavenging effect percentage against extract concentration. Trolox was used an antioxidant standard for comparison of the activity.

Evaluation of total antioxidant activity by phosphomolybdenum method

The total antioxidant capacity of the extracts was evaluated according to the method described by Saravanakumar et al. (2021). An aliquot of 0.3 mL of samples solution was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). In case of blank, 0.5 mL of 45% ethanol was used in place of sample. The tubes were incubated in a boiling water bath at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against blank in UV-2450 spectrophotometer (Shimadzu, Japan). The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance value indicated higher antioxidant activity.

Antimicrobial assay

Microbial strains

All the microorganisms were obtained from the laboratoire de Microbiologie, Department of Biology, University AMAR TELIDHI-LAGHOUAT. One Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) were chosen as test bacteria.

Inoculum preparation

The bacterial strains were grown overnight at 37 °C in Nutrient Agar. Inoculum for the assays was prepared by inoculating three to five colonies from the agar plate culture into 10 mL of nutrient broth and then incubated at 37 °C for 24h. After growing, the microbial suspension was standardized with sterile saline to turbidity equivalent to 0.5 Mc Farland scale (108 CFU/mL for bacteria and 106 CFU/mL for *C. albicans*).

Antimicrobial activity

The antimicrobial tests were carried out by the disc diffusion method (Alpay et al., 2019) using 100

µL of suspension containing 106 per/mL of bacteria, inoculated into Mueller Hinton Agar (Difco). The discs (6 mm) were then impregnated with 100 µL of extract and then placed on the inoculated agar. Petridishes were prepared at 4 °C for 2 h, standard discs of Gentamycin (10 µg/disc), Tetracycline (10 µg/disc) and Ampicillin (10 µg/disc) were used as positive controls for bacteria whereas DMSO discs were used as a negative control. Then, the inoculated plates were incubated at 37±0.1 °C for 24 h for bacterial strains. At the end of the incubation period, the inhibition zones were measured.

Mineral analysis

Each truffle sample was air-dried at room temperature and drying was finished at 105 °C overnight, crushed with a mortar and pestle. About 1 g of truffle dry matter was weighed in a crucible and was ashed at 550 ºC. The ash was then dissolved in 5 mL of HCl (20%) and the solution was transferred to a 50 mL volumetric flask, the final volume was achieved with deionized water and then all was filtered. Analysis of the trace metals was carried out using an atomic absorption spectrophotometer (Vahdani et al., 2017).

Phytochemical investigation

LC-MS/MS

A stock solution of standard compounds were prepared by dissolving them in methanol (1.0 mg/ml) and then diluted to 0.8 ug/ml. after serial dilution of standard mixture, titration levels were separated. The solutions of *Terezia clavery* were prepared as 2.0 mg/ml. finally; they were filtered (0.45µm) and pipette to vials for LC-MS/MS analysis (Atalar et al., 2021, Erenler et al., 2023). The standard compounds are Shikimic acid (1), Gallic acid (2), Protocatechuic acid (3), Epigallocatechin(4), Catechin(5), Chlorogenic acid(6), Hydroxybenzaldeyde (7), Vanillic acid (8), Caffeic acid (9), Syringic acid (10), Caffein (11), Vanillin (12), o-coumaric acid (13), Salicylic acid (14), Taxifolin (15), Resveratrol (16), Polydatine (17), *trans*-ferulic acid (18), Sinapic acid (19), Scutellarin (20), p-coumaric acid (21), Coumarin (22), Protocatehuic ethyl ester (23), Hesperidin (24), Isoquercitrin (25), Rutin (26), Quercetin-3-xyloside (27), Kaempferol-3-glucoside (28), Fisetin (29), Baicalin (30), Chrysin (31), Daidzein (32), *trans*-cinnamic acid (33), Quercetin (34), Naringenin (35), Silibinin (36), Hesperetin (37), Morin (38), Kaempferol (39), Baicalein (40), Luteolin (41), Biochanin A (42), Capcaicin (43), Dihydrocapcaicin (44), and Diosgenin (45).

LC-MS/MS conditions

Quantitative analysis of natural compounds in methanol extract was performed using the spectrophotometer (An Agilent Technologies 1260 Infinity II, jonted 6460 Triple Quad mass spectrophotometre (Spectrometer). A Poroshell 120 EC-C18 (100 mm x 4.6 mm I.D., 2.7 um) column was used 0.1% formic acid and 5.0 mM ammonium formate in water A, 0.1% formic acid, and 5.0 mM ammonium formate in methanol B mobile phase were used. The flow rate was modified to 0.4 mL/min. The gradient program fixed as 15% for 1-12 min, 50% for 12-30 min, 90% for 30-32 min, and 10% for 32-35 min was applied in the mobile phase B. The injection volume was 4.0 uL and the column temperature was 40 °C. The mass ratio in compound to ion (m/z) was determined by negative and positive ionization modes using an electrospray ionization (ESI) source. The capillary voltage was set at 4000 V, nebulizing gas (N) flow was 11 L/min, the nebulizer pressure was 15 psi, and the gas temperature was 300 °C. Each compound's precursor and product ions, collision energies, and cleavage voltage were detected for quantitative analysis as a multiplex reaction control.

Statistical analysis

Data were analyzed with a statistical software program (SPSS 21). Comparisons between multiple numeric data sets were performed using one-way ANOVA followed by Tukey multiple-range test. Results are expressed as mean ± SEM., and statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Extraction yields (%)

Herein, the collected T. claveryi materials were extracted separately with different solvents in descending polarity. The extraction yield (%) was recorded in which the aqueous extract showed the highest yield (40 %), followed by the methanolic extract (19.0 %). The extraction yields of the rest the extracts are in the order: of ethanolic (11.4%), ethyl acetate (10.1 %), acetone (9.4%), and chloroform extract (4.5 %) (Table 1).The high yields of the T. claveryi may be due to their high amounts of phenolic components, soluble sugars, and sugar alcohols.

Table 1. Extraction yields (%) of *T. claveryi* extracts

T. claveryi extracts	Yields (%)
Aqueous	40 %
Methanolic	19 %
Ethanolic	11.4 %
Acetone	9.4 %
Ethyl acetate	10.1 %
Chloroform	4.5 %

Total phenolic and flavonoid contents (TPCs & TFCs)

Polyphenols have a unique chemical structure that combines aromatic properties and a high density of hydroxyl groups, which gives them strong activity as free radical scavenging agents. Accordingly, there is a positive correlation between the presence of polyphenols in the tested extracts and their antioxidant activities (Ghareeb et al., 2018 a; Hamed et al., 2020).The total phenolic and flavonoid contents of the investigated extract are present in Table 2, the TPCs were ranged from 176.67 to 19.92 mg GAE / g DW and the TFCs were ranged from 14.53 to 1.71 mg ER / g DW. The standard curve is shown in Figure 1.

Table 2. Total phenolic and flavonoids contents in *T. claveryi* extracts

T. claveryi extracts	Total phenolic content (mg GAE / g DW)	Total flavonoids content (mg RE / g DW)
Aqueous	176.67 ±6,48^{a}	14.53 ±0,53^{a}
Methanolic	58.89±3,00^{c}	4.89±0,25^{c}
Ethanolic	19.92± 1,47^{d}	1.71± 0,12^{d}
Acetone	117.05 ±11,16^{b}	9.65 ±0,91^{b}
Ethyl acetate	97.86±6,55^{b}	8.08±0,54^{b}
Chloroform	----	----

Each value in the table is represented as mean ± SE (n = 3). GAE, gallic acid equivalents; RE, rutin equivalents; dw, dry weight. Means followed by the same letter are not different according to ANOVA (analysis of variance) ($p < 0.05$). The results are sorted in decreasing order: a > b > c > d.

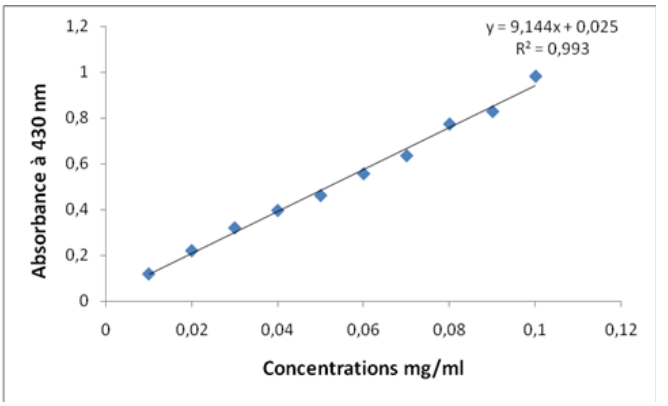

Figure 1. Rutin calibration curve.

In vitro antioxidant activities

Free radicals are highly energetic active molecules that cause many health problems when they penetrate the human cells, it is also considered the main cause of cardiovascular and cancer diseases (Ghareeb et al., 2018 a,b; Khalaf et al., 2021). Natural products have a great role in mitigating the side effects of free radicals and as a safe source of natural antioxidants (Ghareeb et al., 2018 c; Nasr et al., 2018; Sobeh et al., 2018). The antioxidant activities are mainly due to the presence of bioactive ingredients like polyphenolics and others (Bakchiche et al., 2019, 2022; Khalaf et al., 2019; Abdelfattah et al., 2022).

The results presented in Table 3 reveal that the different extracts of *T. claveryi* showed antioxidant activity against DPPH free radical expressed in IC_{50} (mg/ mL)in the order: Aqueous (0.09), methanolic (0.87), ethyl acetate (1.53), ethanolic (2.86), acetone (3.09), and chloroform (6.53) compared to vitamin C (0.001). Also, the extracts exhibited antioxidant effects against ABTS radical with IC_{50} values ranged from 0.24 to 4.24 compared to Trolox (0.033). While in the phosphomolybdate assay the values were ranged from 0.10 to 0.001.

Reviewing the literature revealed that the methanol extract of Turkish *T. claveryi* showed total phenolic content value of 0.0084 mgGAE/mg extract (Taşkın et al., 2018). While, the aqueous methanolic extract of Algerian *T. claveryi* showed total phenolic content value of 15.4±0.11 mg GAE/g) and total flavonoid content value of 12.03 ± 0.27 mg CE/g (Wahiba et al., 2016). On the other side, several previous studies demonstrated the ability of *Terfezia* extracts to scavenge free radicals and as a vital source of antioxidants (Dundar et al., 2012; Taşkın et al., 2018; Wu et al., 2022). The Aqueous extract exhibited an antioxidant effect using DPPH and ABTS assays with an IC50 value of 0.09 and 0.24 mg/ml, respectively. Neggaz et al. (2015) reported that the methanolic extract of Algerian *T. claveryi* showed strong free radical scavenging activity against DPPH radical with IC_{50} value of 8.56 mg/mL. Moreover, the methanolic extract of *T. claveryi* from Algeria exhibited antiradical activity with IC_{50} value of 1.02 mg/mL (Wahiba et al., 2016).

Table 3. Antioxidant activity of the phenolic extracts for *T. claveryi* extracts, expressed in IC_{50} (mg/mL) for DPPH and ABTS assay, VCEAC (vitamin C equivalents mmol of vitamin C/ g dry weight) for Phosphomolybdate assay

T. claveryi extracts	$IC_{50/DPPH}$(mg/mL)	$IC_{50/ABTS}$ (mg/mL)	Phosphomolybdate assay
Aqueous	0.09±0.01[a]	0.24±0.05[a]	0.10±0.02[a]
Methanolic	0.87±0.17[a,b]	0.33±0.09[a]	0.01±0.001[b]
Ethanolic	2.86±0.07[c]	4.24±0.21[d]	0.03± 0.006[b]
Acetone	3.09±1.01[c]	2.01±0.09[c]	0.005± 0.0005[c]
Ethyl acetate	1.53±0.07[b]	1.30±0.28[b]	0.007± 0.001[c]
Chloroform	6.53±0.23[d]	-------	0.001±0.002[c]
Vitamin C	0.001±5.98E^{-05}[a]	-------	-------
Trolox	-------	0.003±0.0002[a]	-------

Values are presented as mean ± standard error of three replicates. Values followed by the same letter within a column are not statistically different according to Tukey's multiple range test.

***In vitro* antimicrobial activity**

The rapid spread of infectious microbial diseases as a result of bad habits and also as a result of the fierce resistance of some microbial strains to current antibiotics encouraged scientists to search for alternative natural sources to obtain promising and safe antibiotics (Ghareeb et al., 2015; Mohammed et al., 2019; Abdel-Motleb et al., 2022; El-Shazly et al., 2022). Natural extracts have a long history in eliminating infectious diseases, especially their activities against pathogenic microbial strains (El-Neekety et al., 2016; Ghareeb et al., 2016a,b; Hathout et al., 2016; Saad et al., 2017; Abdel-Aziz et al., 2018; Elkhouly et al., 2021a,b).

Our current findings showed that the different extracts of *T. claveryi* showed variable antimicrobial activities against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* with inhibition zones ranged from 5.80 to 21.91 mm, from 4.43 to 13.53 mm and from 4.53 to 6.67 mm, respectively. The methanolic extract exhibited the most potent antimicrobial activity against *Pseudomonas aeruginosa* with inhibition zone value of 21.91 mm followed by the aqueous extract with inhibition zone value of 15.79 mm. Also, the methanol and aqueous extracts showed the strongest effects against *Escherichia coli* with inhibition zones value of 13.53 and 12.68 mm, respectively. All extracts showed a moderate effect against *Staphylococcus aureus* (Table 4).

Among the tree different types of antibiotics used in the study, Gentamycin has wide range of impact on all the three species of human pathogenic bacteria. The maximum zone of inhibition was observed against *Pseudomonas aeruginosa*. The maximum zone of inhibition was obtained using Gentamycin (25.12 mm) against *Pseudomonas aeruginosa*, (16.35 mm) against *Escherichia coli* and (11.85 mm) against *Staphylococcus aureus* whereas the minimum zone of inhibition was exhibited in Ampicillin (8.25 mm) against *Staphylococcus aureus* (Table 4). Altogether, the antibiotics Gentamycin, Tetracycline and Ampicillin have higher antimicrobial activity on the selected test organisms.

Previous reports revealed that different *Terfezia* species showed strong antimicrobial activities against various pathogenic microbial strains due to the presence of several bioactive compounds (İnci and Kirbağ, 2018; Neggaz et al., 2019; Ghareeb et al., 2020). Dib-Bellahouel and Fortas (2019) reported that the extract of the culture filtrate of *T. claveryi* inhibits the growth of *Gliocladium roseum*, *Candida albicans* and *Staphylococcus aureus* with inhibition

zone values of 11, 23 and 10.8 mm, respectively. The acid-soluble protein extract of *T. claveryi* exhibited an antimicrobial effect against gram-positive and gram-negative phytopathogenic bacteria including *Pseudomonas corrugate (CFBP 5454), Pseudomonas mediterranea (CFBP 5447T), Pectobacterium carotovorum (CFBP 2046T), Pectobacterium syringae (PVCT 28.3.1), Clavibactermic higanensis (PVCT 156.1.1) and Xanthomona svesicatoria (CFBP 2537T)* with inhibition zone values of 0.38, 0.40, 0.33, 0.52, 1.26 and 1.88 cm, respectively (Gargano et al., 2017). Moreover, the aqueous extract of *T. claveryi* showed antibacterial activity against three strains of *P. aeruginosa* including *P. aeruginosa* ATCC14028, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 9027 with inhibition zone values of 21.0, 28.0 and 19.0 mm, respectively (Saddiq et al., 2016). Alhussaini et al (2016) reported that the methanol extract of Saudi *T. claveryi* showed antibacterial activity against some bacterial strains including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zone values of 5.0, 4.0, 5.0 and 4.0 mm, respectively. Additionally, the aqueous extract of *T. claveryi* showed antibacterial effect against *Staphylococcus aureus (ATCC 6538), Staphylococcus epidermidis (ATCC 12228), Streptococcus faecalis, Escherichia coli (ATCC 29425), Pseudomonas aeruginosa (ATCC 9027), Proteus vulgaris (ATCC 8427) and Klebsiella pneumonia (ATCC 13883)* with inhibition zone values of 19.0, 18.0, 17.0, 15.33, 20.33, 15.33 and 14.66 mm, respectively (Aldebasi et al., 2013). In addition, it is noted for its antimicrobial and antioxidant activities, T. claveryi is strongly affected by the nature of the solvents used.

Table 4. Diameter of zone of inhibition of *T. claveryi* extracts

T. claveryi extracts	Gram (-)		Gram (+)
	Pseudomonas aeruginosa ATCC 23853	*Escherichia coli* ATCC 23822	*Staphylococcus aureus* ATCC 23823
Zone of inhibition (mm)			
Aqueous	15.79	12.68	5.67
Methanolic	21.91	13.53	4.53
Acetone	7.67	5.43	4.88
Ethyl acetate	10.96	7.45	6.67
Chloroform	5.80	4.43	4.65
Ampicillin	23.85	15.36	8.25
Gentamycin	25.12	16.35	11.85
Tetracycline	24.54	14.21	10.51

Mineral content

Different *Terfezia* species contain important nutritional components for the growth of the human body, especially the mineral elements (Dundar et al., 2012; Wahiba et al., 2016; Khlaif et al., 2021).Herein, the truffle extracts showed a varied mineral content including macroelements like Na (3293.01mg·kg^{-1}dw) and K (21092.19mg·kg^{-1}dw). Also, there are micro-elements like Fe (16.45 mg·kg^{-1}dw), Cu (22.80 mg·kg^{-1}dw), and Ni (27.88 mg·kg^{-1}dw). While, other elements like Pb, Cd, and Co are absent (Table 5).

Table 5. Mineral content of truffle extracts

	Truffle extracts
	Macroelements (mg·kg^{-1}dw)
Na	3293.01±95.04^{b}
K	21092.19±96.67^{a}
	Micro-elements (mg·kg^{-1}dw)
Fe	16.45± 0.008^{c}
Cu	22.80± 0.27^{c}
Ni	27.88±0,40^{c}
Pb	ND
Cd	ND
Co	ND

Values are presented as mean ± SE (n = 3). Different letters above the average bars denote significant differences at $p < 0.05$ - Tukey's test.

Phytochemical investigation using LC-MS/MS

LC-MS/MS analysis was performed on the methanol extract of *T. claveryi* in order to determine the chemical components compared to 46 standards (Table 6). The analysis showed the presence of six compounds *viz*., caffein, scutellarin, hesperidin, isoquercitrin, rutin, quercetin-3-xyloside, and morin. These compounds were identified in the extract at contents equal to 0.9229, 0.0322, 22.4629, 1.2009, 38.0089, 2.5611, and 0.0358 mg/g, respectively. Rutin and hesperidin were dominant in the extract (Figures 2, 3 and Table 7). Our current findings are in agreement to some extent with the previous reports. Al-Atassi et al. (2022) stated that LC-MS/MS analysis of 70% ethanolic extract of *T. claveryi* led to identification of 14 compounds belonging to phenolic acids and flavonoids including p-hydroxy benzoic acid, syringic acid, *trans*-cinnamic acid, p-coumaric acid, gallic acid, homogentisic acid, protocatechuic acid, vanillin, ferulic acid, rutin, vanillic acid, apigenin, catechin, and hesperidin. In the same context, UPLC-ESI-MS analysis of the *T. claveryi* aqueous extract led to identification of some phenolic, organic and fatty acids including succinic acid, coumaric acid, vanillic acid, scopoletin, palmitic acid, *trans*-vaccenic acid, and stearic acid as well as protocatechuic aldehyde (Abu-Odeh et al., 2022). Additionally, Vahdani et al (2017) reported that HPLC investigation of *T. claveryi* led to detection of several phenolic compounds including gallic acid, catechin, chlorogenic acid, rutin, p-coumaric acid, hesperedin, and eugenol.

Table 6. Phenolic content of T. claveryi extract using LC/ESI-MS-MS

Peak No.	Compound name	RT (min)	Content (mg/g)
1	Shikimic acid	1.406	ND
2	Gallic acid	3.292	ND
3	Protocatechuic acid	5.537	ND
4	Epigallocatechin	6.878	ND
5	Catechin	6.880	ND
6	Chlorogenic acid	7.498	ND
7	Hydroxybenzaldeyde	7.791	ND
8	Vanillic acid	7.860	ND
9	Caffeic Acid	7.875	ND
10	Syringic acid	8.383	ND
11	Caffein	8.404	0.9229
12	Vanillin	8.631	ND
13	o-coumaric acid	9.628	ND
14	Salicylic Acid	9.316	ND
15	Taxifolin	9.921	ND
16	Resveratrol	9.545	ND
17	Polydatine	9.975	ND
18	Trans-ferulic acid	10.323	ND
19	Sinapic acid	10.424	ND
20	Scutellarin	11.289	0.0322
21	p-coumaric acid	11.427	ND
22	Coumarin	11.525	ND
23	Protocatehuic ethyl ester	11.496	ND
24	Hesperidin	12.094	22.4629
25	Isoquercitrin	12.110	1.2009
26	Rutin	12.078	38.0089
27	Quercetin-3-Xyloside	12.676	2.5611
28	Kaempferol-3-glucoside	13.443	ND
29	Fisetin	13.461	ND
30	Baicalin	13.637	ND
31	Chrysin	14.204	ND
32	Daidzein	14.263	ND
33	Trans-cinnamic acid	14.281	ND
34	Quercetin	15.074	ND
35	Naringenin	15.184	ND
36	Silibinin	15.211	ND
37	Hesperetin	15.983	ND
38	Morin	15.845	0.0358
39	Kaempferol	16.599	ND
40	Baicalein	17.135	ND
41	Luteolin	18.002	ND
42	Biochanin A	17.926	ND
43	Capcaicin	18.245	ND
44	Dihydrocapcaicin	18.817	ND
45	Diosgenin	23.601	ND

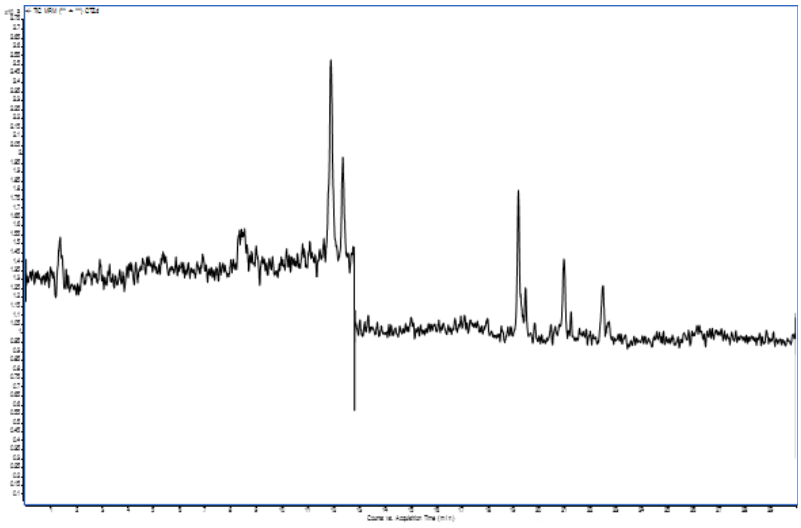

Figure 2. LC-MS chromatogram of *T. claveryi* extract

Table 7. Detected compounds in the methanol extract using LC-MS/MS

Peak No.	Name	RT (min)	Content (mg/g)	Classification	Reported bioactivity	References
1	Caffein	8.404	0.9229	Alkaloids	Anti-inflammatory, antiapptotic and antioxidant	Saud and Salamatullah, 2021
2	Scutellarin	11.289	0.0322	Flavonoids	Antioxidant, antimicrobial, anti-rheumatoid, and anti-coagulation	Vesaghhamedani et al., 2023
3	Hesperidin	12.094	22.4629	Flavonoids	Antiradical, antioxidant, and anti-cancer	Öngün et al., 2021
4	Isoquercitrin	12.110	1.2009	Flavonoids	Antioxidant	Razavia et al., 2009
5	Rutin	12.078	38.0089	Flavonoids	Antioxidant	-
6	Quercetin-3-xyloside	12.676	2.5611	Flavonoids	Antioxidant	-
7	Morin	15.845	0.0358	Flavonoids	Antioxidant Anti-lipid peroxidation, antifungal, anticancer, and treatment diabetes mellitus	Hussain et al., 2014; Yang and Lee, 2012; Nazir et al., 2021

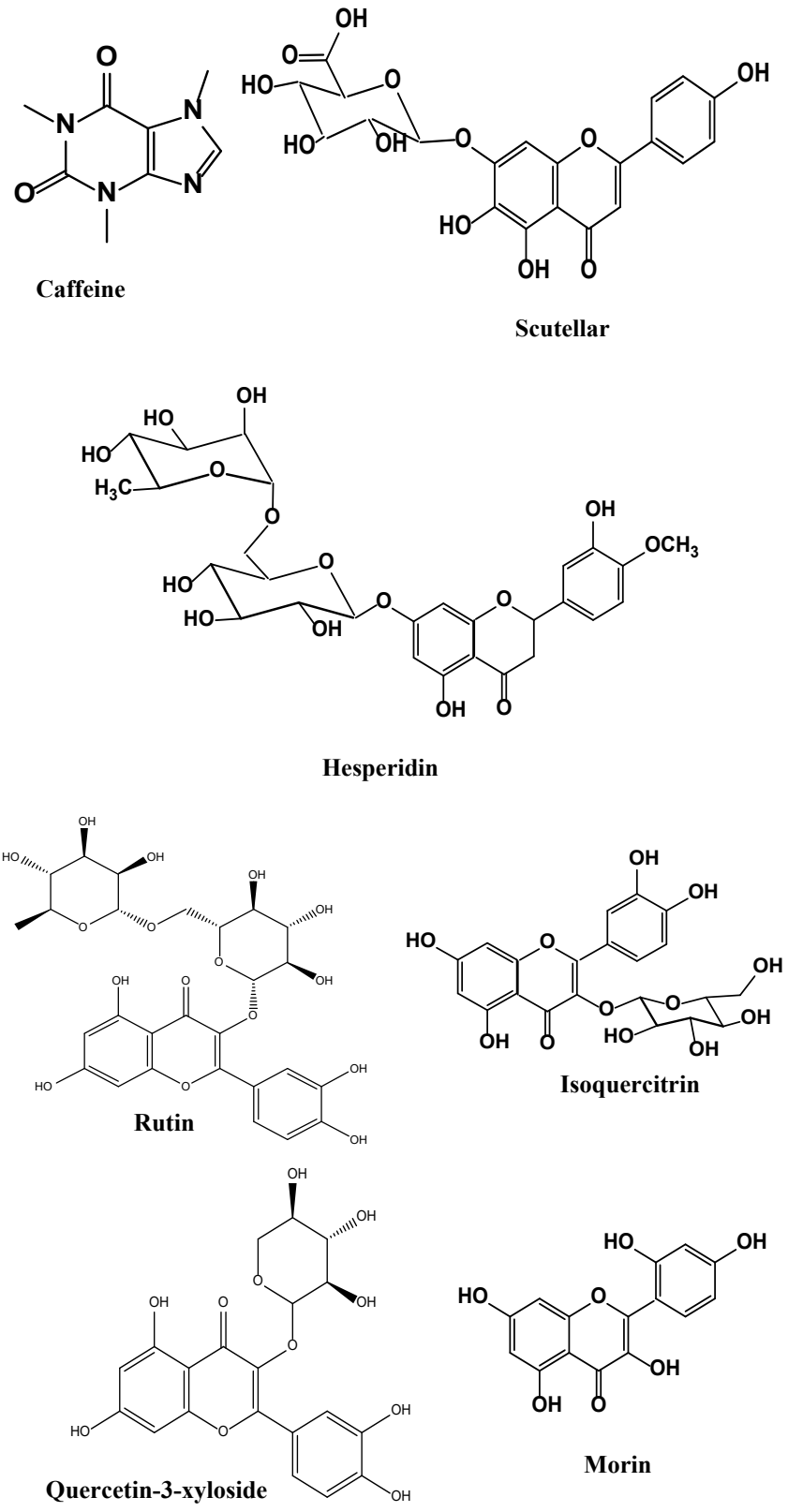

Figure 3. Chemical structures of the identified compounds using LC-MS/MS

CONCLUSION

In this work, we discovered the antioxidant and antimicrobial potential of different solvent extracts of *T. claveryi*. Also, the study aimed to the determination of their mineral content, accompanied by the identification of the chemical components of the methanol extract using LC-MS/MS technique, which led to the identification of some phenolic compounds which are known for its multiple biological activities. To sum up, *T. claveryi* extracts are considered as promising sources of natural antioxidant and antimicrobial agents. Additionally, its high nutritional content also makes it a promising source for nutritional supplements.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Hadjira GUENANE: Plants collection, perform extraction, antioxidant activity evaluation, and writing-original draft preparation. Boulanouar BAKCHICHE: writing-original draft preparation and supervision. Ramazan ERENLER and Ilyas YILDIZ: Perform LC-ESI-MS/MS analysis. Asmaa S. MOHAMED and Maha A.M. El-SHAZLY: Formal analysis, investigation, data curation, visualization, writing-original draft preparation, review and editing,

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