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Research Article

Antioxidant properties and chemical composition of white truffle *Tirmania nivea* and its host plant *Helianthemum lippii*

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Abstract

This study aims to determine the phytochemical contents and the antioxidant activity of the white truffle *Tirmania nivea* (Desf.) Trappe, and its host plant *Helianthemum lippii* (L.) Dum. Cours. Standard procedures were applied for phytochemical analysis, including the determination of proximate compositions, mineral elements, and various secondary metabolites. The antioxidant activities were assessed using the 1,1-diphenyl-2-picrylhydrazyl, azinobis benzo thiazoline sulphonic, and ferric reducing antioxidant power assays. Chemical analysis revealed the presence of carbohydrate content, which has the highest value in the truffle and its host plant. The carbohydrate content is the highest in *T. nivea* compared with *H. lippii*. Crude lipids showed the lowest value in *T. nivea* and *H. lippii.* Potassium is the highest concentration of a macro-element in the truffle and the host plant. Iron is the highest concentration of micro-element in the truffle and host plant. Phenolics had the highest concentration of secondary metabolisms, but tannins had the lowest value in the truffle and its host plant. Concerning the antioxidant activity, we found that hexane extract of *T. nivea* and *H. lippii* has high inhibitory percentages equivalent to IC_{50} 134.8 and 119.9 µg/mL, respectively for DPPH, 128.84, 111.21 µmol Fe^{2+}/mg, respectively for FRAP, and 121.39 and 107.22 µg/mL, respectively for ABTS. The study proved that the aqueous extract of the truffle is much richer than the host plant in proximate constituents, minerals elements, and secondary metabolites; in addition, the truffle has a tremendous antioxidant capacity than the host plant, which leads us forward to introduce it for medical use.

Keywords: antioxidant activity; host plant; phytochemical properties; truffle

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Introduction

Most land plants form symbiotic relationships with various species of mycorrhizal fungi (Lee *et al*., 2020). The truffle usually forms a mycorrhizal association mainly on the roots of different species of the genus *Helianthemum* (Gabr *et al*., 2023). Truffles are ectomycorrhizal with a symbiotic root relationship and the mycelium forms a symbiotic relationship with its host plant in the family Cistaceae as *Helianthemum* (Le Tacon *et al*., 2014 and Mouffouk *et al*., 2023). The genus *Helianthemum* serves as the host plant for truffles, and consists of approximately 110 species worldwide, found in Europe, America, North Africa and Central Asia (Mabberly, 1997). As well as, several species of the plant *Helianthemum lippii, H. aegyptiacum H. tuberia H. guttatum, H. salicifolium, H. ladyfolium, and H. almeriense* have been reported as hosts of desert truffles. Most of the truffles are morphologically varied due to the diversity of the natural area, types of soil, and climate fluctuations (Mouffouk *et al*., 2023).

Truffles are underground ascomycete fungi that grow 5 to 10 cm underground. Taxonomically edible truffles belong to the Tuberaceae and Pezizaceae families and the Pezizales order (El Enshasy *et al*., 2013). Desert truffles grow in arid and semi-arid areas. Generally, truffles belong to the genus *Tirmania* and *Terfezia* (Bradai *et al*., 2015). In general, truffles tend to be firm, dense and woody in comparison with mushrooms that are soft and fragile. White truffles include *Tirmania nivea, Tuber borchii, T. magnatum, T. latisporum, T. japonicum, T. maculatum,* and *T. oregonense* (Wang and Marcone, 2011 and Lee *et al*., 2020). Black truffles include *Terfezia claveryi, Tuber melanosporum, T. aestivum, T. brumale, T. uncinatum, T. himalayense,* and *T. indicum* (Lee *et al*., 2020).

Partnerships formed between host plants and mycorrhizal truffles are among the most widespread symbioses. Mycorrhizal truffles can form associations with roots and participate in nutrient exchange with host plants (Brundrett and Tedersoo, 2018). Mycorrhizal truffles enhance host plant access to mineral nutrients and help facilitate the movement of these nutrients within the host plants. The truffles form intricate networks of external mycelium that can extend beyond plant root systems. In the soil, they forage for nitrogen, phosphorus, sulfur, and trace elements with water (Kakouridis et al., 2022). These nutrients are exchanged for photosynthetically derived carbohydrates and fats from the host plant and delivered to the roots. The symbiosis is a fundamental part of host plant nutrition, symbiotic truffles have the ability of to extract nutrients from minerals and transfer these nutrients to their host plants. About 80 % of phosphorus and over 20% of nitrogen can be transferred to the host plant via mycorrhizal pathways (Ji and Bever, 2016). The roots of the host plant release carbon that stimulates the host plant to absorb nutrients from the truffle and in turn, the resources become mutual (Bogar *et al*., 2022). Sometimes, one or other of the partners can receive more than they provide (Brundrett, 2002).

Desert truffles have antioxidant activities (Al-Laith, 2010; Alhussaini et al, 2016). *T. nivea* contains key flavor components such as sulfur volatiles, but are less intense in aroma and flavor than European truffles (Mustafa *et al*., 2020). The medicinal plants are important sources of bioactive phytochemical compounds like saponins, flavonoids, tannins, steroids and glycosides which are known to have antimicrobial, antibacterial and antioxidant properties (Temerk *et al*., 2017; Abdel-alim *et al*., 2023). Plant extracts contain phytochemicals such as flavonoids, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates, showing antibacterial, antiviral, and antifungal properties. These properties led to their use in Egyptian folk medicine for treating diabetes and gastrointestinal tract ailments (El-Chaghaby *et al*., 2024). Several researchers have been investigating how the antioxidant potential of a plant can be related to its anticancer effects (Adia *et al*., 2016).

The bioactive compounds are responsible for the antioxidant properties. Flavonoids, alkaloids, and polyphenols are antioxidant agents that could reduce oxidative stress imposed by free radicals (Kaurinovic and Vastag, 2019). Natural antioxidants may play a crucial role in the development of effective anticancer treatments (Adebiyi *et al*., 2017). This significant attribute could be more effective in the treatment of cancer

than drugs that are target-specific in their mode of action (Okaiyeto and Oguntibeju, 2021). Thus, the study aimed to evaluate the phytochemical and antioxidant properties of white truffle (*T. nivea*) and its host plant (*H. lippii*). This study focuses on the antioxidant properties of white truffles and their host plants, due to their nutritional and therapeutic values. Antioxidants are compounds that, even in slight concentrations, can delay, control, or prevent oxidative processes. These processes can lead to the deterioration of food quality or the onset and progression of degenerative diseases in the body. Evaluating the antioxidant capacity of medicinal plants, such as truffle and its host plants, is crucial. This analysis involves various methods and activities aimed at inhibiting oxidation through these antioxidant compounds.

Materials and Methods

Biological material

Truffle samples of *T. nivea* and fresh leaves of the host plant *H. lippii* were collected randomly from the study area located on the northern coast of the Mediterranean, and identified in the Zagazig University Herbarium, where they are kept (Figure 1). The young leaves were air-dried and transported to the laboratory for extraction. The truffle samples were dried in an oven at 35-40 °C and then ground to a fine powder using a blender. The extracts were prepared using different solvents like hexane, ethyl acetate, ethanol, methanol and water solvents. Each sample solvent is dissolved in its solvent; i.e., the hexane extract was dissolved in hexane, the ethyl acetate extract was dissolved in ethyl acetate, the ethanol extract was dissolved in ethanol, the methanol extract was dissolved in methanol, and the water extract was dissolved in water. The supernatant was obtained after filtration (Neggaz and Fortas, 2013).

(a) (b)

Figure 1. The white truffle *T. nivea* (a) and its host plant *H. lippii* (b) growing under natural conditions

The white Truffle *T. nivea* and its host plant *H. lippii* are identified based on visual characteristics such as morphology and color, identification of species was according to Täckholm (1974) and Boulos (2009).

Tirmania nivea

Ascocarps: often lobed or subglobose pyriform, with a short, smooth pedicel that can grow up to 4–8 cm in diameter and a white to whitish cream color.

Peridium: yellowish milky white, 1.5-2 mm thick.

Gleba: solid, meaty, white, and somewhat marbled, with veins that range in width from 1.8 to 4.9 mm.

Helianthemum lippii

H. lippii is a perennial herb with upright flowering stems that reach 15.0 to 25.0 cm tall. The stems are woolly with close, star-shaped hairs and some that spread out. The leaves are opposite, elongated ovals with curled edges. The upper side has a dense, woolly covering, while the underside is lighter with spreading hairs. The top leaves are longer than the spaces between the stem sections, while the bottom leaves are shorter.

Petiole is sessile, acuminate and consistent. Flowers pendulous in a panicle with 3-30 flowered. Pedicels without articulation, outer and Inner sepals broadly ovate, mucronate, tips of sepals erect in flower, the surface adpressed-stellate, bifurcate and simple hairy.

Petals yellow, obovate, undulate, longer than sepals. Stamens numerous, contain multiseriate, filament, anthers. Style doubly curved, Stigma capitate. Spreading-stellate-hairy capsules with longer, denser, simple bifurcate-hairy tips.

Determination of proximate compositions

Five grams of each fresh sample were dried in an oven at 105 °C until a constant weight was obtained. The loss in weight was then calculated as a percentage of moisture. After determining the moisture content, the samples were placed in a muffle furnace and heated to 600 °C for two hours. After cooling, the residue was weighed, and the weight was calculated as a percentage of ash. Two grams of powder from each sample were boiled in 200 mL of 1.25 % sulphuric acid, filtered, and then boiled with 200 mL of 1.25 % NaOH. The resulting residue was filtered and dried at 100 °C until a constant weight was obtained, and the weight was estimated as crude fibre (Latimer, 2023).

To determine the total nitrogen content, take 0.03 g of dried powder and heat it with 0.5 g catalyst, 2 mL of ammonia, and 1 mL of distilled water. Treat the resulting solution with 15 mL of 40% NaOH and measure the optical density at 450 nm. The crude protein content of each sample can be calculated by multiplying the total nitrogen by a factor of 6.25. Samples extracted in ether should be filtered, and the residue then dried, cooled, and weighed to determine the percent lipid content. The organic matter can be calculated by subtracting the ash content from the % dry matter. Next, subtract the sum of the percentages of all other proximate components from 100 to determine the total carbohydrate content (Latimer, 2023).

Estimation of mineral elements

The truffle and the host plant samples were digested with concentrated HNO_3 (65 %) and 60 % $HClO_4$ (20 mL HNO_3 + 5 mL $HClO_4$). Iron, Zinc, manganese, copper, magnesium, phosphorus, and cobalt were determined by atomic absorption spectrometer (CCT, China). Potassium, calcium, and sodium were determined by an atomic emission spectrometer (CCT, China) (Latimer, 2023).

Determination of some secondary metabolisms

Determination of phenolics, saponins, and tannins was performed on the powdered specimens, while the aqueous extract was analysed for alkaloids and flavonoids (Sofowara, 1993).

Total phenolic content

Total phenolic compounds were measured according to the Folin-Ciocalteu method. 10 µL of 1 mg/mL of ethanol extracts were added to 1 mL distilled water and 10 µL of 2 N Folin-Ciocalteu reagent was added. Then, 20 µL of 2% (w/v) of sodium carbonate solution was added, and incubated in the dark at room temperature for 3 hours until firm blue color. The absorbance was measured at 760 nm using a spectrophotometer. Quercetin was used as a standard reference (Khoddami *et al*., 2013).

Total alkaloids determination

1 mL of hydrogen chloride was mixed with 3 mL of each extract in a test tube. The mixture was heated for 20 minutes, filtered, and then cooled. About 3 drops of Mayer's reagent were added to 1 mL of the extract. If a creamy precipitate appeared, it indicated the presence of an alkaloid. To further test for alkaloids, 100 mL of 10% acetic acid was added to the ethanol extract, followed by the addition of NH_4OH until precipitation appeared. The resulting residue was then dried and weighed (Trease and Evans, 1989; Yadav and Agarwala, 2011).

Total flavonoids content

Add 1 mL of 10% sodium hydroxide to 2 mL of the extract to detect flavonoids, indicated by the appearance of a yellow color. Next, mix 2 g of powder samples with 5 mL of 80% methanol, centrifuge the mixture, and concentrate the resulting supernatant using reduced-pressure rotary evaporation (Tungmunnithum *et al*., 2018).

Total saponins content

Mix 0.5 grams of dried sample with 5 mL of distilled water in a test tube and shake it vigorously. The presence of saponins is indicated by the formation of stable foam. Next, mix 20 grams of each sample with 200 mL of 20% ethanol. Heat the mixture at 90 °C until it is reduced to 40 mL, then filter it. Add 20 mL of diethyl ether, followed by 60 mL of n-butanol, and then 10 mL of 5% aqueous NaCl. Heat the mixture and evaporate it until a constant weight is achieved. The saponin content can then be calculated as a percentage (Obadoni and Ochuko, 2002).

Total tannins content

To test for tannins, 1 g of dried powder samples was taken and boiled in 40 mL of water. The mixture was then filtered. After that, a few drops of 0.1 % ferric chloride were added, which resulted in a brownish-green color. Then the methanol extraction was centrifuged and 1 mL of the supernatant to 10 mL of vanillin hydrochloride reagent. A standard curve was prepared using 0- 100 µg of tannic acid. After 20 minutes, the optical density of the color was measured at 500 nm (Sivananth and Manickam, 2008).

Determination of antioxidant activity

DiPhenyl Picryl Hydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the truffle and the host plant extracts was determined by a DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay (Bondet *et al*., 1997). Briefly, the reaction mixture contains 1 mL of 0.1 mM solution of DPPH (Creative Egyptian Biotechnologists (CEB), Egypt) in methanol, mixed with 2 mL of the aqueous extracts; the mixture was incubated for 30 min in the dark at room temperature. Controls were prepared by mixing 1 mL of DPPH solution with double distilled water. Triplicate samples were prepared and ascorbic acid was used as a standard. The absorbance was measured at λ517 nm by Systronics Controller Based Visible Spectrophotometer (UNICO, Japan). The percent scavenging activity of each extract against DPPH radicals was calculated at inhibitory concentration IC_{50} for dose-response, % DPPH inhibition (I %) using the following equation:

% DPPH radical scavenging activity= [1- (A sample- A control)] × 100

A sample is the test extract solution absorbance and A control is the control absorbance

Azinobis Benzo Thiazoline Sulphonic (ABTS) radical scavenging assay

The antioxidant activity of the desert truffle and the host plant extracts was determined by the ABTS radical scavenging activity method. The ABTS stock solution was prepared at ABTS (CEB, Egypt) aqueous solution (7 mM) with 2.45 mM aqueous solution of potassium persulfate (CEB, Egypt) in equal quantities,

stored in a dark room at temperature for 16 h before use. Prepared the working solution of ABTS by stock solution is diluted in methanol, until giving an absorbance 0f 0.70 ± 0.02 at 734 nm. Add 2.0 mL of working solution of ABTS to 1 mL of the sample aqueous extract and incubate in the dark for 10 min. The control solution was prepared by adding 2.0 mL of working solution of ABTS to 1 mL of double distilled water and then mixing them. Adjust spectrophotometer (UNICO, Japan) at 734 nm by measuring a blank solution. The antioxidant butylated hydroxytoluene BHT (CEB, Egypt) was used as a standard solution. Samples were prepared and measured in triplicate (Re et al. 1999).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of the extracts of the tested truffle and its host plant was assessed by FRAP assay (Halvorsen *et al*., 2002). Briefly, 2.3 mL of the FRAP reagent (CEB, Egypt) was added to 0.7 mL of the aqueous extracts, and incubated in the dark, for 30 min at 37 °C. The developed color was measured at 590 nm using a spectrophotometer, normalizing to the blank solution, regarding the authenticity of ascorbic acid. Results were expressed in mg of equivalent ascorbic acid in the extract as (AAE/ mL).

Statistical analysis

Statistical Analysis of antioxidant activity by DPPH, FRAP, and ABTS assays was performed in triplicate. Analysis of variance and significant differences and representation of experimental data as mean ± SD based on hypothesis testing.

Results

Phytochemical analysis

Determination of proximate constituents in white truffle and its host plant

Dry matter, moisture, total ash, crude fiber, crude lipid, total nitrogen, crude protein, organic matter, and total carbohydrate content were estimated and shown in Table 1.

Table 1. Proximate constituents in the white truffle and their host plant

Plant materials (%)	Truffle *Tirmania nivea*	Host plant *Helianthemum lippii*
Dry matter	32.11± 1.98	26.42 ± 1.75
Moisture	68.34 ± 2.81	65.11 ± 2.51
Ash	16.11 ± 0.36	14.12 ± 0.21
Fiber	14.99 ± 0.15	11.41 ± 0.13
Lipid	6.99 ± 0.19	5.51 ± 0.13
Total nitrogen	8.25 ± 0.15	7.61 ± 0.11
Protein	40.14 ± 2.58	35.87 ± 2.11
Organic matter	32.98 ± 1.89	28.11 ± 1.25
Carbohydrates	53.87 ± 3.51	47.51 ± 3.11

The results showed that the plant materials content of the white truffle, *T. nivea*, is higher than that of its host plant, *H. lippii*; however, the moisture content represented the highest proportion of them. The white truffle contains a higher percentage of carbohydrates, protein, and organic matter (53.87%, 40.14%, and 32.98%, respectively) compared to its host plant, which contains these materials in lower proportions (47.51%, 35.87%, and 35.87%, respectively). Lipid had the lowest plant material in the white truffle and its host plant (Table 1).

Elementary analysis of white truffle and its host plant

The concentrations of the macro (Na^+, K^+, Ca^{++}, Mg^{++}, P) and micro-elements (Fe, Cu, Mn, Zn, and Co) are estimated in the truffle and the host plant (Table 2). The results showed that potassium is the highest concentration macro-element in the truffle and the host plant with 15.14 and 11.14 mg/ g dry weight, respectively. Phosphorus recorded high concentration in truffle with 5.58 mg/ g dry weight and in the host plant with 3.98 mg/ g dry weight. The magnesium concentration ranged from 4.83 mg/ g dry weight in the truffle to 3.21 mg/ g dry weight in the host plant. Calcium showed a narrow range value in truffle and the host plant ranged from 3.85 to 2.58 mg/ g dry weight, respectively. Sodium is the lowest concentration recorded in the truffle with 1.84 mg/ g dry weight and in the host plant with 1.24 mg/ g dry weight (Table 2).

Table 2. Mineral elements concentration of the white truffle and its host plant

Elements	Elements (mg/g dry weight)	Truffle *Tirmania nivea*	Host plant *Helianthemum lippii*
Macro-elements (mg/ g dry weight)	Na^+	1.84 ± 0.16	1.24 ± 0.15
	K^+	15.14 ± 1.91	11.14 ± 1.82
	Ca^{++}	3.85 ± 0.25	2.58 ± 0.21
	Mg^{++}	4.83 ± 0.28	3.21 ± 0.21
	P	5.58 ± 0.29	3.98 ± 0.52
Micro-elements (mg/ g dry weight)	Fe	4.25 ± 0.18	2.98 ± 0.28
	Cu	2.28 ± 0.02	1.59 ± 0.14
	Mn	1.11 ± 0.03	0.74 ± 0.02
	Zn	3.21 ± 0.11	2.41 ± 0.05
	Co	1.58 ± 0.08	0.99 ± 0.03

Iron concentration is the highest concentration of micro-element in the truffle and host plant with 4.25 and 2.98 mg/ g dry weight, respectively. Zinc recorded high concentrations in the truffle and the host plant with 3.21 and 2.41 mg/ g dry weight, respectively. Cobalt and copper concentrations showed a narrow range of variation that ranged from 1.58 and 2.28 mg/ g dry weight in truffle, to 0.99 and 1.59 mg/ g dry weight in the host plant, respectively. Manganese is the lowest concentration of micro-element recorded in the truffle with 1.11 mg/ g dry weight and in the host plant with 0.74 mg/ g dry weight. This showed that mineral elements are higher in desert truffles than in their host plant (Table 2).

Quantitative analysis of some Secondary metabolisms

The comparative study of truffle *T. nivea* and the host plant *H. lippii* extracts revealed that both were high in the composition of phenolics, saponins, and tannins. However, the truffle exhibited the highest significant values compared to the host plant (Table 3).

The results showed that phenolics are the highest concentration with 6.54 mg/g dry weight in truffle, with 5.48 mg/g dry weight in the host plant, followed by saponins with 4.65 mg/g dry weight in truffle, and 3.28 mg/g dry weight in the host plant. Alkaloids recorded with 4.25 mg/g dry weight in truffle but recorded 3.14 mg/g dry weight in the host plant. Flavonoids recorded 3.58 mg/g dry weight in the truffle but recorded 2.54 mg/g dry weight in the host plant. Tannins recorded the lowest value in the truffle and their host plant with 3.12 and 2.17 mg/g dry weight, respectively (Table 3).

Table 3. Concentration of secondary compound in the white truffle and their host plant

Secondary compound (mg/g DW)	Truffle *Tirmania nivea*	Host plant *Helianthemum lippii*
Phenolics	6.54 ± 0.19	5.48 ± 0.11
Alkaloids	4.25 ± 0.13	3.14 ± 0.81
Flavonoids	3.58 ± 0.17	2.54 ± 0.02
Saponins	4.65 ± 0.14	3.28 ± 0.14
Tannins	3.12 ± 0.09	2.17 ± 0.08

Antioxidant activity

The antioxidant activity of the truffle and the host plant extracts was assessed by the DPPH, FRAP and ABTS Free Radical scavenging activity assay (Table 4).

Table 4. Antioxidant activity of different extracts of *Tirmania nivea (*white truffle) and its host

	Samples	DPPH (IC_{50} µg/mL)	FRAP (µgmol Fe^{2+}/mg)	ABTS (IC_{50} µg/mL)
T. nivea	Hexane	134.8 ± 9.01 a	128.84 ± 9.31 k	121.39 ± 9.23 a
	Ethyl acetate	119.78 ± 8.25 b	111.21 ± 8.22 g	104.11 ± 7.21 a
	Ethanol	128.93 ± 8.91 c	121.10 ± 5.21 f	112.79 ± 6.20 f
	Methanol	113.50 ± 6.01 d	107.04 ± 7.25 a	101.63 ± 7.22 r
	Water	99.81 ± 7.81 e	92.24 ± 7.56 k	89.30 ± 5.23 m
	Ascorbic acid	68.3 ± 2.30 f	61.7 ± 2.01 k	54.4 ± 3.22 b
H. lippii	Hexane	119.9 ± 8.11 a	111.21 ± 6.02 d	107.22 ± 5.01 a
	Ethyl acetate	104.82 ± 8.21 o	96.99 ± 7.25 a	89.83 ± 4.25 b
	Ethanol	112.11 ± 2.21 n	106.58 ± 4.11 n	98.78 ± 5.11 c
	Methanol	99.50 ± 5.02 p	91.44 ± 5.21 b	83.78 ± 9.10 c
	Water	84.91 ± 3.30 h	77.29 ± 4.41 c	71.51 ± 7.46 d
	Ascorbic acid	53.3 ± 1.12 b	46.7 ± 1.11 v	40.4 ± 2.21 a

The results of independent experiments (DPPH, FRAP and ABTS) are different significantly at $P < 0.05$, and are expressed as mean ± SD. and also (a, b, c, d, etc. is the significant letters).

DPPH radical scavenging activity

The results displayed as the most effective methods DPPH, with IC_{50} values are illustrated in Table 4. All extractions of two samples, which desert truffle as *T. nivea*; and its host plant *H. lippii* have amounts significant of DPPH radical scavenging activity. The strongest DPPH radical scavenging activity with an IC_{50} of *T. nivea* is the hexane extraction of 134.8 µg/mL, followed by ethanol, ethyl acetate, and water extracts with IC_{50} values of 128.93, 119.78, and 99.81µg/mL respectively. The lowest DPPH with an IC50 value of *T. nivea* extraction was methanol which showed 113.50 µg/mL, while the positive control ascorbic acid showed 68.3 µg/mL. The strongest DPPH radical scavenging activity with an IC_{50} of *H. lippii* was hexane extraction of 119.9 µg/mL, followed by ethanol, ethyl acetate, and water extracts with an IC_{50} of 112.11, 104.82, and 84.91 µg/mL, respectively. The lowest DPPH with an IC_{50} of *H. lippii* extraction was methanol which showed 62.10µg/mL, while the positive control ascorbic acid showed 53.3µg/ mL. By the DPPH method, white truffle *T. nivea* showed the highest scavenging activity than the host plant *H. lippii*. There are significant differences between the aqueous extracts of white truffle and its host plant. Hexane extraction was the strongest DPPH of desert truffle and its host plant. The weakest DPPH of desert truffle and its host plant was methanol extraction (Figure 2).

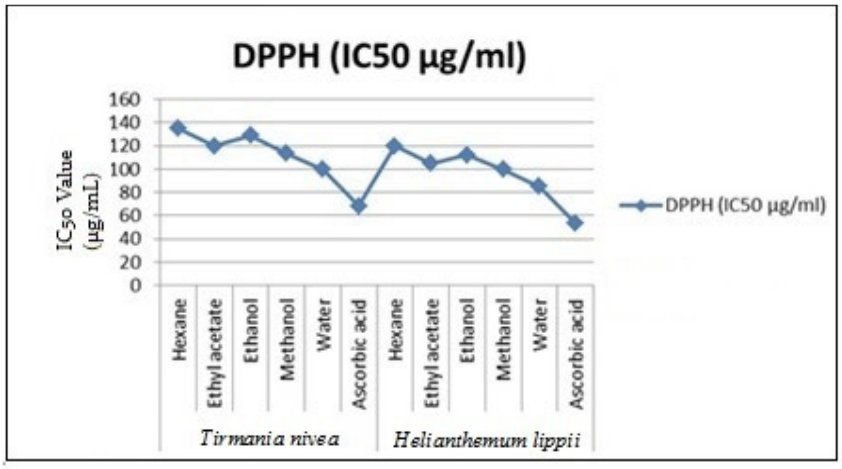

Figure 2. DPPH inhibition of the white truffle *T. nivea* and their host plants *H. lippii* extracts

FRAP Ferric reducing antioxidant power

FRAP is a direct examination to measure the antioxidant properties of two sample extractions, which desert truffle as *T. nivea*; and its host plant *H. lippii* (Table 4). The peak FRAP trait of *T. nivea* was hexane extract with 128.84 µmol Fe^{2+}/mg, followed by ethanol, ethyl acetate and water extractions with 121.10, 111.21, 92.24 µmol Fe^{2+}/mg, respectively. Methanol extract showed lower FRAP with 107.04 µmol Fe^{2+}/mg.

The peak FRAP trait of *H. lippii* was hexane extract with 111.21 µmol Fe^{2+}/mg, followed by ethanol, ethyl acetate and water extractions with 106.58, 96.99 and 77.29 µmol Fe^{2+}/mg, respectively. Methanol extract showed lower FRAP with 91.44 µmol Fe^{2+}/mg. The reducing ability of the aqueous extracts of the white truffle *T. nivea* and its host plant *H. lippii* have been assessed by the FRAP method. Truffle was recorded with the highest reducing ability than host plant. A significant difference was observed in aqueous extracts of truffle and the host plant. Hexane was the highest extraction of the white truffle and its host plant, but methanol was the lowest extraction of the white truffle and its host plant (Figure 3).

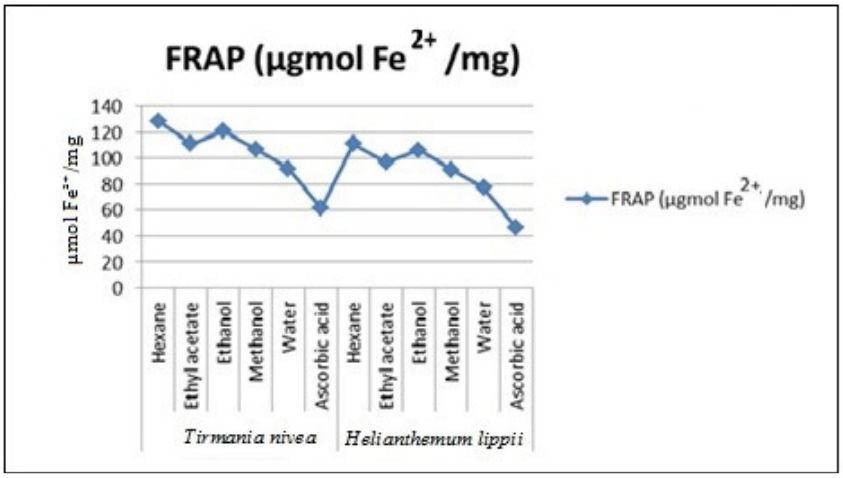

Figure 3. Ferric reducing antioxidant activity of the white truffle *T. nivea* and its host plant *H. lippii* extracts

ABTS scavenging assay

The ABTS scavenging activity was to determine the total antioxidant capacity and the activity of antioxidants in two samples, which desert truffle as *T. nivea*, and its host plant *H. lippii*. Hexane extracts of *T.*

nivea and H. lippii have the maximum inhibition activity with IC50 values of 121.39, and 107.22 µg/ mL; also, methanol extracts of *T. nivea* and *H. lippii* have the maximum inhibition activity with IC50 values of 101.63, and 83.78 µg/ mL. The ethanol, ethyl acetate and water extracts of *T. nivea* have 112.79, 104.11, and 89.30 µg/ mL, respectively. Also, ethanol, ethyl acetate and water extracts of *H. lippii* have 98.78, 89.83 and 71.51 µg/ mL, respectively (Table 4). So, there is a marked correlation between DPPH, ABTS and FRAP results referring to the significance of these antioxidants for the chemical properties of two samples, desert white truffles as *T. nivea*; and its host plant *H. lippii*. Truffle showed the highest ABTS radical scavenging activity than the host plant. Based on hypothesis testing, a significant difference between all the aqueous extracts of truffle and the host plant was observed. Ethanol is the highest extraction of white truffle and its host plant, but methanol was the lowest extraction of both (Figure 4).

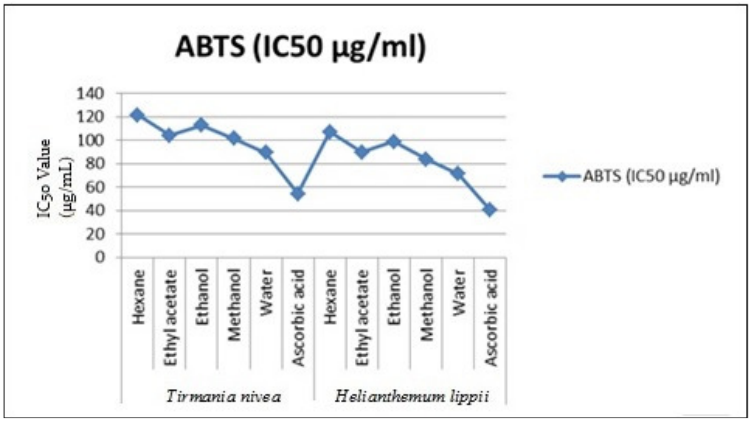

Figure 4. Inhibition of ABTS of the white truffle *T. nivea* and host plant *H. lippii* extracts

Discussion

Medicinal plants are an important source of bioactive phytochemical compounds that serve as antioxidants. According to our study, the hexane extracts of *Tirmania nivea* (white truffle) and its host plant, *Helianthemum lippii,* were generally found to be more effective as potential antioxidant agents than other extracts. This conclusion was drawn from tests conducted using three methods to determine antioxidant activity: DPPH, ABTS, and FRAP. The aqueous extracts of white truffle and their host plant displayed remarkable antioxidant activity, as revealed from the DPPH assay. This is due to DPPH being one of the most effective methods for assessing the concentration of radical scavenging materials (Adam *et al*., 2021). The hexane extracts of truffle *T. nivea* showed the highest scavenging activity and more antioxidant activity than the host plant *H. lippii*. This activity could be attributed to all extractions of the truffles having significant amounts of radical scavenging activity (Allen and Bennett, 2021). Also, there is a high correlation between secondary compounds and various antioxidant assays in the desert truffles (Allen and Bennett, 2021). The white truffles contain secondary components such as alkaloids, phenolics, flavonoids, saponins and tannins (Gioacchini *et al*., 2005). These secondary compounds are often called antioxidants because they can scavenge free radicals. They function as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers (Temerk *et al*., 2017; El-Chaghaby *et al*., 2024). In addition, these have antioxidant effects such as scavenging peroxy radicals and chelating ferric ions (Al-Laith, 2010; Üstün *et al*., 2018). The secondary compounds are secondary metabolites that are produced in the desert white truffle *T. nivea* more than its host plant *H. lippii*. Due to enables the truffle to adapt to its local environment with the host plant and enhance its ability to survive in its environment and overcome threats in its niche, whereas the soil has a rich nature pathogenic, so the root

often produces antioxidant phytochemicals compounds and transfers to symbiotic truffles via mycorrhizal pathways (Bogar *et al*., 2022; Bitwell *et al*., 2023).

Truffles are rich in nutrients such as carbohydrates, proteins, fats, fiber, lipids, ash, and minerals (Wang and Marcone, 2011; Lee *et al*., 2020). The primary compounds are primary metabolites that are produced in the desert truffle *T. nivea* more than the host plant *H. lippii*. Due to growth regulators in symbiosis relation, and cell wall components this leads to normal growth and development (Awuchi, 2019; Bitwell *et al*., 2023). Although the presence of active substances in the host plant is less than that found in the desert truffle. *Helianthemum* species used for hepapatoprotective (Morita *et al*., 2003), gastrointestinal disorders, constipation (Rahuman *et al*., 2000), blood-cutting (Baytop, 1999), anti-inflammatory (Tung *et al*., 2008), antioxidant (Allen and Bennett, 2021), antiulcerogenic (Chang *et al*., 2001), antiparasitic (Parang *et al*., 1997), antimicrobial (Cheng *et al*., 2006), analgesic (Rubio-Moraga *et al*., 2013), cytotoxic (Mouffouk *et al*., 2023), vasodilator medicines (Ziaei et al. 2011), wound healing and burn treatments since ancient times (Gökşena *et al*., 2017).

White truffle *T. nivea* called Zubaidi is rich in carbohydrates and proteins, in addition to potassium present in greatest amounts followed by phosphorus, but other minerals (copper, sodium, calcium, iron, magnesium, manganese, and cobalt) were present in low, that agree with our results (Hawkins *et al*., 2023). Truffles such as *T. nivea*, *T. boudieri*, and *T. melanosporum* contain phenolic compounds and a limited quantity of lipids, which agree with our results (Villares *et al*., 2012). But, *T. nivea* does not contain cadmium, mercury, arsenic, oxalate and cyanide as antinutrient compounds (Bouatia *et al*., 2018; Bitwell *et al*., 2023). Truffle *Tuber pseudohimalayense* showed phenols have a high content of secondary compounds, but *Tuber subglobosum* showed a high content of flavonoids (Yan *et al*., 2017). There are differences in the nutritional composition details of truffles in previous studies with our findings, due to truffles varying from species to species and area to area and having different geographical origins (Lee *et al*., 2020). Al-Naama *et al*. (1988) conducted a study on *T. nivea* collected from Saudi Arabia and reported results that were lower than our study in carbohydrates (21.53%), ash (4.90%), and protein content (13.84%), except phosphorous element was higher than our study, about 25.50%. Also, Al-Naama *et al*. (1988) collected *T. nivea* from Egypt and showed that the protein content is lower than our study, about 16.30%.

Hussain and Al-Ruqaie (1999) reported that *T. nivea* contains crude fibre and fat in a very close percentage to our study, about 13.02% and 7.42%, respectively. However, phosphorous, cobalt elements and moisture content are higher than in our study, about 11.54%, 5.04%, and 75.27%, respectively (Table 5). But, ash and protein content are lower than in our study, about 5.40% and 27.18, respectively. Also, some minerals were lower than in our study, such as Ca (0.83%), Mg (1.90%), Na (0.68%), K (12.70%), Fe (1.30%), Cu (0.19%), Mn (0.33%), Zn (0.49%).

Table 5. Illustration of the proportion of plant material, some elements, and minerals of *T. nivea* reported by Hussain and Al-Ruqaie (1999)

Plant materials					**Elements**	
Crude fibre	Fat	Moisture content	Ash	Protein	Phosphorous	Cobalt
13.02	7.42	75.27	5.40	27.18	11.54	5.04
Minerals						
Ca	Mg	Na	K	Fe	Cu	Mn	Zn
0.83	1.90	0.68	12.70	1.30	0.19	0.33	0.49

Akyüz (2013) indicated that *T. nivea* contains crude fiber (7.4%) higher than in our study, but crude ash (5.4%) and crude protein (27.2%) lower than in our study. Another study conducted on *T. nivea* by Al-Ruqaie (2002) showed that crude ash (1.30%) and crude protein (65.8%) are higher than in our study, but the

crude fiber (1.10%) showed lower than in our study. El Enshasy *et al*. (2013) demonstrated that the chemical composition of truffle (*T. nivea*) has richness in fiber (7-13%) and fatty acids (3-7.5%), which is a very close percentage to our study. Also, they showed proteins (20-27%) that were lower than in our our research, but carbohydrates (60%) that were higher than in our study. Also, they indicated *T. nivea* contains diverse mineral content such as Si, K, Na, Ca, Mg, Mn, Fe, Al, Cu, and Zn.

A desert truffle genera *Tirmania* exhibited lower protein (8-29 g/100 g DW) than in our study, also, ash content (5.1-5.3 g/100 g DW) was lower than in our study. However, carbohydrate and fat content are a very close percentage to our study (58-83 g/100 g DW) (4-7 g/100 g DW), respectively (Bouatia *et al*., 2018). The desert white truffle *T. nivea* exhibited relatively lower total phenolic content than our findings (1.39 g GAE/100 g fresh mass (FM), but contained a high number of flavonoids (74.52 mg GAE/g extract) and tannins (19.78 mg catechin equivalent (CE)/g extract) (Lee et al. 2020), this dissimilar our study.

The biochemical composition of truffles encompasses multiple nutritional and medicinal benefits, potent antioxidant activity, and metabolites with anti-carcinogenic, anti-depressant, anti-inflammation, antimicrobial, and immunomodulatory properties (Lee *et al*., 2020; Wu *et al*., 2021). The carbohydrates isolated from the truffle had immunomodulating and antitumor activity (Guo *et al*., 2022). The water extract of truffles plays a significant role in radical scavenging activity and treatment of eye infections without side effects, such as Prophet Muhammad (Peace Be Upon Him) mentioned in prophetic medicine (Owaid, 2018).

Hexane exhibits the highest radical scavenging activity in the desert truffle, *T. nivea*, and its host plant. However, the phenolic compounds were evaluated using ethanol extracts from the truffle and the host plant. This approach is more effective for selectively obtaining phenolics with high antioxidant capacity from medicinal plants (Temerk *et al*., 2017; Bitwell *et al*., 2023). In contrast, Yan *et al*. (2017) indicated that methanol is the highest radical scavenging activity in traditional edible truffles *Tuber latisporum*, *T. subglobosum,* and *T. pseudohimalayense*, collected from China.

The inhibition percent of DPPH quenching of the dried truffles ranged between 24.5 and 69.2%, with an average of 30.6% (Akyüz, 2013). It seems that the radical scavenging activity of *T. nivea* might be different than other truffles. The antioxidant activity of truffles depends on their relation with the type, the ascocarp size, harvest time, the host plant, and the ecosystems. Also, the antioxidant attributes of the truffles may be affected by their nature and the extent of association with their host root associate *Helianthemum* spp. (Munteanu and Apetrei, 2021). The higher scavenging effect of the *T. nivea* than its host plant is due to the correlation with phenolics present in *T. nivea* host plant, which agrees with previous studies (Doğan and Aydın, 2013; Lee *et al*., 2020). DPPH is considered the most effective method for evaluating the concentration of radical-scavenging materials (Pokorny *et al*., 2001). Al-Laith (2010) conducted DPPH scavenging activity on the Saudi truffle *T. nivea* and showed that the hexane extract has the highest DPPH radical scavenging activity at about 69%, while the methanol extract has the lowest DPPH radical scavenging activity at about 24%, and these agree with our results. Also, our result agrees with the study reported that the hexane extract of *T. boudieri* has a high DPPH activity of 22.24% (Akyüz, 2013).

Another study was conducted by Sallam *et al*. (2022) on different concentrations of ethyl extract of *Tirmania* sp. and *Terfezia* sp. collected from Egypt. The study showed that *Tirmania sp.* and *Terfezia sp*. had high DPPH scavenging antioxidant activity values of 91.56 µg/mL and 93.75 µg/mL, respectively; these findings agree with our results. Another study was conducted by Wu *et al*. (2021) on black truffle *T. indicum* collected from China. They showed that black truffles exhibited the highest DPPH and ABTS values and potent anti-inflammatory effects; this study agrees with our results.

Hsu *et al*. (2021) conducted DPPPH, ABTS, and FRAP radical scavenging activity on ethanol and water extracts of white truffles. They showed that the ethanol extract of white truffle recorded the highest values of DPPH, ABTS, and FRAP at 92.77, 91.28, and 1.556%, respectively. They exhibited the DPPH, ABTS, and FRAP of water extract at 91.35, 90.18, and 1.456%, respectively; these agree with our results.

Ethyl acetate extraction showed the highest DPPH radical scavenging activity reported by Dahham *et al*. (2018) in their study on *T. claveryi* with an IC_{50} of 121.84µg/ mL. The hexane extraction recorded high DPPH radical scavenging activity with an IC_{50} of 87.21 µg/ml. The methanol and water extract were recorded with an IC50 of 69.50 µg/ml and 57.9µg/ml, respectively. However, ethanol has the lowest DPPH radical scavenging activity with an IC_{50} of 52.10µg/ mL. Also, other studies conducted on *T. claveryi* showed the ethyl acetate extracts have the highest DPPH with IC_{50} of 57.73 µg/mL (Kıvrak, 2014). These previous studies showed that the DPPH activity was affected more by the extraction solvents. Still, these are in contrast with our result, where the hexane extract is the highest DPPH radical scavenging activity, followed by ethanol, ethyl acetate, methanol, and water extracts in descending order, which disagrees with previous results conducted by (Kıvrak, 2014; Dahham *et al*., 2018).

FRAP is considered a direct assay to measure antioxidant properties in various samples (Halvorsen *et al*., 2002). Our results agree with Dahham *et al*. (2018) reported that the hexane extract of *T. claveryi* has the highest FRAP with 105.8 µmol Fe^{2+}/mg, and the methanol extract has the lowest FRAP with 66.04 µmol Fe^{2+}/mg. In contrast, the study conducted by Al-Laith (2010) on desert truffle *T. nivea* by using FRAP antioxidant activity showed that FRAP has good antioxidant activity with values ranging from 15.41 to 3.51 mmol/100g dw, and these disagree with our results.

Our results agree with Dahham *et al*. (2018), where the hexane extract of *T. claveryi* exhibited the highest FRAP radical scavenging activity with 105.8 µmol Fe^{2+}/mg. Also, the methanol extract showed the lowest FRAP radical scavenging activity with 66.04 µmol Fe^{2+}/mg. However, our results do not agree with Dahham *et al*. (2018), where the ethyl acetate showed the highest ABTS radical scavenging activity with 133.71 µg/ mL, and the ethanol is the lowest ABTS radical scavenging activity with 64.76 µg/ mL. These activities might seem paradoxical (Bondet *et al*., 1997), but the extraction method and solvent used significantly influence the solubility and nutritional composition of bioactive compounds (Rahman *et al*., 2011). Shah and Modi (2015) conducted DPPH, ABTS and FRAP on water extract of three truffles as *G. lucidum, L. edodes* and *A. bisporus* collected from India. They exhibited high values of DPPH radical scavenging activity at 5 mg/ mL concentration that 68.10, 41.16 and 35.12%, respectively. Also, they showed high values of ABTS radical scavenging activity at 5 mg/ mL concentration that 72.22, 51.5 and 49.38%, respectively. In addition to, they reported high values of FRAP radical scavenging activity at 5 mg/ mL concentration, and the values increased with increasing the concentration of truffle extract.

Conclusions

This study aimed to determine the phytochemical and antioxidant activity of the desert truffle, *T. nivea*, and its host plant *H. lippii* using three simple spectrophotometric methods "DPPH, ABTS, and FRAP". The white truffle has antioxidant activity higher than its host plant. The antioxidant properties of the white truffles and its host plant are a good indication for use as a treatment for trachoma. However, further research into the mechanisms of the bioactive compounds from this extract may lead to the discovery of chemotherapeutic drugs. Therefore, it is suggested that white truffle has the potential to be developed as a new natural antioxidant agent that may benefit patients suffering from free radicals-related disease and patients who undergo nuclear medicine procedures. The present study shows that desert truffles represent great interest in food, economic, and therapeutic values. The white truffle is richer in protein, minerals, and carbohydrates than its host plant and has at the same time therapeutic values.

Authors' Contributions

Conceptualization: AAZ, HMS, AMA and MAY; Formal analysis: AAZ; Investigation: AAZ, HMS, AMA and MAY; Methodology: AAZ, HMS, AMA and MAY; Visualization: AAZ, HMS, AMA and MAY; Writing - original draft: AAZ, HMS, AMA, MAY; Review and editing: MAY and KA; Data curation: AAZ, NA, AR, DA and AE. Funding acquisition: NA, AR, DA, AE. Writing review and editing: AAZ, HMS, AMA, MAY, NA, AR, DA, AE and KA.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdel-alim M, Serag M, Moussa H, Elgendy M, Mohesien M, Salim N (2023). Phytochemical screening and antioxidant potential of *Lotus corniculatus* and *Amaranthus viridis*. Egyptian Journal of Botany 63(2):665-681. *https://doi.org/10.21608/ejbo.2023.158720.2118*

Adam Z, Razali R, Arapoc DJ, Aziz AHA, Marsiddi NA (2021). DPPH radical scavenging and Folin-Ciocalteu assays: simple and reliable methods to quantify antioxidant activity and total phenolic content. Nuclear Technical Convention 8:1-8. *https://www.researchgate.net/publication/353463146*

Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z (2017). In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Suef University Journal of Basic and Applied Sciences 6:10-14. *https://doi.org/10.1016/j.bjbas.2016.12.003*

Adia MM, Emami SN, Byamukama R, Faye I, Borg-Karlson AK (2016). Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants. Journal of Ethnopharmacology 186:14-19. *https://doi.org/10.1016/j.jep.2016.03.047*

Akyüz N, Altman RB, Blanchard SC, Boudker O (2013). Transport dynamics in a glutamate transporter homologue. Nature 502(7469):114-118. *https://doi.org/10.1016/j.bpj.2012.11.2983*

Alhussaini MS, Saadabi AM, Hashim K, Al-Ghanayem AA (2016). Efficacy of the desert truffle *Terfezia claveryi* to cure trachoma disease with special emphasis on its antibacterial bioactivity. Trends in Medical Research 11:28-34. *https://doi.org/10.3923/tmr.2016.28.34*

Al-Laith AAA (2010). Antioxidant components and antioxidant/antiradical activities of desert truffle (*Tirmania nivea*) from various Middle Eastern origins. Journal of Food Composition and Analysis 23(1):15-22. *https://doi.org/10.1016/j.jfca.2009.07.005*

Allen K, Bennett JW (2021). Tour of truffles: aromas, aphrodisiacs, adaptogens and more. Mycobiology 49(3):201-212. *https://doi.org/10.1080/12298093.2021.1936766*

Al-Neama MM, Ewaz JO, Nema JH (1988). Chemical constituents of Iraqi truffles. Iraqi Journal of Agricultural Sciences 6:51-56. *https://doi.org/19880390311*

Al-Ruqaie IM (2002). Effect of different treatment processes and preservation methods on the quality of truffles: I. Conventional methods (drying/freezing). Pakistan Journal of Biological Sciences 26(1-2):177-182. *https://www.cabidigitallibrary.org/doi/full/10.5555/19880390311*

Awuchi CG, Igwe VS, Echeta CK (2019). The functional properties of foods and flours. International Journal of Advanced Academic Research 5(11):139-160. *https://www.academia.edu/download/61281991/ijaar-ste-v5n11-nov19-p1620191120-120155-x5po6j.pdf*.

Bitwell C, Indra SS, Luke C, Kakoma MK (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African 19:15-85. *https://doi.org/10.1016/j.sciaf.2023.e01585*

Bogar LM, Tavasieff OS, Raab TK, Peay KG (2022). Does resource exchange in ectomycorrhizal symbiosis vary with competitive context and nitrogen addition?. New Phytologist 233:1331-1344. *https://doi.org/10.1111/nph.17871*

Bondet V, Brand-Williams W, Berset CLWT (1997). Kinetics and mechanisms of antioxidant activity using the DPPH Free radical method. LWT-Food Science and Technology 30(6):609-615. *https://doi.org/10.1006/fstl.1997.0240*

Bouatia M, Touré HA, Cheikh A, Eljaoudi R, Rahali Y, Idrissi OMB, Draoui M (2018). Analysis of nutrient and antinutrient content of the truffle (*Tirmania pinoyi*) from Morocco. International Food Research Journal 25:174-178.

Boulos L (2009). Flora of Egypt. Checklist revised annotated (Ed). Al-Hadara Publishing, Cairo, Egypt, pp 410.

Bradai L, Bissati S, Chenchouni H, Amrani K (2015). Effects of climate on the productivity of desert truffles beneath hyper-arid conditions. International Journal of Biometeorology 59:907-915. *https://doi.org/10.1007/s00484-014-0891-8*

Brundrett MC, Tedersoo L (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytologist 220:1108-1115. *https://doi.org/10.1111/nph.14976*

Chang ST, Chen PF, Wang SY, Wu HH (2001). Antimite activity of essential oils and their constituents from *Taiwania cryptomerioides*. Journal of Medical Entomology 38(3):455-457. *https://doi.org/10.1603/0022-2585-38.3.455*

Chen Y, Xie MY, Nie SP, Li C, Wang YX (2008). Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. Food Chemistry 107:231-241. *https://doi.org/10.1016/j.foodchem.2007.08.021*

Cheng SS, Liu JY, Hsui YR, Chang ST (2006). Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*). Bioresource Technology 97(2):306-312. *https://doi.org/10.1016/j.biortech.2005.02.030*

Dahham SS, Al-Rawi SS, Ibrahim AH, Majid ASA, Majid AMSA (2018). Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle *Terfezia claveryi*. Saudi Journal of Biological Sciences 25(8):1524-1534. *https://doi.org/10.1016/j.sjbs.2016.01.031*

Doğan HH, Aydın S (2013). Determination of antimicrobial effect, antioxidant activity and phenolic contents of desert truffle in Turkey. African Journal of Traditional, Complementary and Alternative Medicines 10:52-58. *https://doi.org/10.4314/ajtcam.v10i4.9*

El Enshasy H, Elsayed EA, Aziz R, Wadaan MA (2013). Mushrooms and truffles: historical biofactories for complementary medicine in Africa and in the middle east. Evidence-Based Complementary and Alternative Medicine 2013:1-10. *https://doi.org/10.1155/2013/620451*

El-Chaghaby GA, Mohammed FS, Rashad S, Uysal I, Koçer O, Lekesiz Ö, Sevindik M (2024). Genus *Hypericum*: General %%. Egyptian Journal of Botany 64(1):1-26. *https://doi.org/10.21608/ejbo.2023.217116.2378*

Gabr ME, El-Ghandour HA, Elabd SM (2023). Prospective of the utilization of rainfall in coastal regions in the context of climatic changes: case study of Egypt. Applied Water Science 13(1):19. *https://doi.org/10.1007/s13201-022-01835-9*

Gioacchini G, Cardinali M, Maradonna F, Funkenstein B, Mosconi G, Carnevali O (2005). Hormonal control of the IGF system in the sea bream ovary. Annals of the New York Academy of Sciences 1040(1):320-322. *https://doi.org/10.1196/annals.1327.051*

Gökşena N, Demircib B, Baldemirc A, Koşara M (2017). Essential oil composition of *Helianthemum canum* (L.) Baumg. (Cistaceae) growing in Turkey. Asian Society of Pharmacognosy 1(1):5-10. *http://www.pharmacognosyasia.com/Files/Other/AJPV1I1p0510.pdf*

Guo Q, Huang X, Kang J, Ding H, Liu Y, Wang N, Cui SW (2022). Immunomodulatory and antivirus activities of bioactive polysaccharides and structure-function relationship. Bioactive Carbohydrates and Dietary Fibre 27:100301. *https://doi.org/10.1016/j.bcdf.2021.100301*

Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Blomhoff R (2002). A systematic screening of total antioxidants in dietary plants. The Journal of Nutrition 132(3):461-471. *https://doi.org/10.1093/jn/132.3.461*

Hawkins HJ, Cargill RI, Van Nuland ME, Hagen SC, Field KJ, Sheldrake M, Kiers ET (2023). Mycorrhizal mycelium as a global carbon pool. Current Biology 33(11):R560-R573. *https://doi.org/10.1016/j.cub.2023.02.027*

Hsu JY, Chen MH, Lai YS, Chen SD (2021). Antioxidant profile and biosafety of white truffle mycelial products obtained by solid-state fermentation. Molecules 27(1):109. *https://doi.org/10.3390/molecules27010109*

Hussain G, Al-Ruqaie IM (1999). Occurrence, chemical composition, and nutritional value of truffles: an overview. Pakistan Journal of Biological Sciences 2(2):510-514. *https://doi.org/10.3923/pjbs.1999.510.514*

Ji B, Bever JD (2016). Plant preferential allocation and fungal reward decline with soil phosphorus: Implications for mycorrhizal mutualism. Ecosphere 7:e01256. *https://doi.org/10.1002/ecs2.1256*

Kakouridis A, Hagen JA, Kan MP, Mambelli S, Feldman LJ, Herman DJ, Firestone MK (2022). Routes to roots: direct evidence of water transport by arbuscular mycorrhizal fungi to host plants. New Phytologist 236(1):210-221. *https://doi.org/10.1111/nph.18281*

Kaurinovic B, Vastag D (2019). Flavonoids and phenolic acids as potential natural antioxidants. IntechOpen, London, pp 1-20. *https://doi.org/10.5772/intechopen.83731*

Khoddami A, Wilkes MA, Roberts TH (2013). Techniques For Analysis Of Plant Phenolic Compounds. Molecules 18(2):2328-2375. *https://doi.org/10.3390/molecules18022328*

Kıvrak I (2014). Analytical methods applied to assess chemical composition, nutritional value and in vitro bioactivities of *Terfezia olbiensis* and *Terfezia claveryi* from Turkey. Food Analytical Methods 1-15. *https://doi.org/10.1007/s12161-014-0009-2*

Latimer GW (2023). Official Methods of Analysis of AOAC INTERNATIONAL (22). Oxford University Press.

Le Tacon F, Marçais B, Courvoisier M, Murat C, Montpied P, Becker M (2014). Climatic variations explain annual fluctuations in French Périgord black truffle wholesale markets but do not explain the decrease in black truffle production over the last 48 years. Mycorrhiza 24:115-125. *https://doi.org/10.1007/s00572-014-0568-5*

Lee H, Nam K, Zahra Z, Farooqi MQU (2020). Potentials of truffles in nutritional and medicinal applications: a review. Fungal Biology and Biotechnology 7:1-17. *https://doi.org/10.1186/s40694-020-00097-x*

Mabberley DJ (1997). The plant-book: a portable dictionary of the vascular plants (second edition). Cambridge university press, pp 107-177.

Magalhaes LM, Segundo MA, Reis S, Lima JL (2008). Methodological aspects about in vitro evaluation of antioxidant properties. Analytica Chimica Acta 613:1-19. *https://doi.org/10.1016/j.aca.2008.02.047*

Morita T, Jinno K, Kawagishi H, Arimoto Y, Suganuma H, Inakuma T, Sugiyama K (2003). Hepatoprotective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/d-galactosamine-induced liver injury. Journal of Agricultural and Food Chemistry 51(6):1560-1565. *https://doi.org/10.1021/jf020946n*

Mouffouk S, Mouffouk C, Mouffouk S, Haba H (2023). Medicinal, pharmacological and biochemical progress on the study of genus *Helianthemum*: A review. Current Chemical Biology 17(3):147-159. *https://doi.org/10.2174/2212796817666230227112835*

Munteanu IG, Apetrei C (2021). Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences 22(7):3380. *https://doi.org/10.3390/ijms22073380*

Mustafa AM, Angeloni S, Nzekoue FK, Abouelenein D, Sagratini G, Caprioli G, Torregiani E (2020). An overview on truffle aroma and main volatile compounds. Molecules 25(24):5948. *https://doi.org/10.3390/molecules25245948*

Neggaz S, Fortas Z (2013). Tests of antibiotic properties of algerian desert truffle against bacteria and fungi. Life Sciences 7:259-266.

Obadoni BO, Ochuko PO (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 8(2):203-208. *https://doi.org/10.4314/gjpas.v8i2.16033*

Okaiyeto K, Oguntibeju OO (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. International Journal of Environmental Research and Public Health 18(11):5988. *https://doi.org/10.3390/ijerph18115988*

Owaid MN (2018). Bioecology and uses of desert truffles (Pezizales) in Middle Eastern. Walailak Journal of Science and Technology 15(3):179-188. *http://orcid.org/0000-0001-9005-4368*

Parang K, Wiebe LI, Knaus EE, Huang JS, Tyrrell DL, Csizmadia F (1997). *In vitro* antiviral activities of myristic acid analogs against human immunodeficiency and hepatitis B viruses. Antiviral Research 34(3):75-90. *https://doi.org/10.1016/S0166-3542(96)01022-4*

Pokorny J, Yanishlieva N, Gordon M (2001). Antioxidants in Food: Practical Applications. Woodhead Publishing 7-21.

Rahman NN, Nama MM, Al-Rawi SS, Ibrahim AH, Kadir MOA (2011). Comparison of nutritional composition between palm kernel fibre and the effect of the supercritical fluid extraction on its quality. Procedia Food Science 1:1940-1945. *https://doi.org/10.1016/j.profoo.2011.09.285*

Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B (2000). Effect of *Feronia limonia* on mosquito larvae. Fitoterapia 71(5):553-555. *https://doi.org/10.1016/S0367-326X(00)00164-7*

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9-10):1231-1237. *https://doi.org/10.1016/S0891-5849(98)00315-3*

Rubio-Moraga A, Argandona J, Mota B, Perez J, Verde A, Fajardo J, Gomez-Navarro J, Castillo-Lopez R, Ahrazem O, Gomez-Gomez L (2013). Screening for polyphenols, antioxidant and antimicrobial activities of extracts from eleven *Helianthemum* taxa (Cistaceae) used in folk medicine in South-Eastern Spain. Journal of Ethnopharmacology 148:287-296. *https://doi.org/10.1016/j.jep.2013.04.028*

Sadasivam S, Manickam A (2008). Biochemical Methods. Third Edition, New Age International Publishers, pp 203-204.

Saddiq AA, Danial EN (2012). Assessment of phenolic content, free radical-scavenging capacity and antimicrobial activities of Truffle *claveryi*. Wulfenia Journal 19:403-422. *file:///C:/Users/WIN7/Downloads/25Dramna%20(1).pdf*

Sallam F, El-Mokadem M, AI Mekawey A, Saker E (2022). Egyptian truffles as a source of antimicrobial and antioxidant agents. Journal of Scientific Research in Science 39(2):49-61. *https://doi.org/10.21608/jsrs.2022.275787*

Shah P, Modi HA (2015). Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. International Journal for Research in Applied Science and Engineering Technology 3(6):636-641. *https://www.researchgate.net/publication/307464470*

Sofowora A (1993). Recent trends in research into African medicinal plants. Journal of Ethnopharmacology 38(2-3):197-208. *https://doi.org/10.1016/0378-8741(93)90017-Y*

Sreeramulu D, Raghunath M (2010). Antioxidant activity and phenolic contents of roots, tubers and vegetables commonly consumed in India. Food Research International 43:1017-1020. *https://doi.org/10.1016/j.foodres.2010.01.009*

Täckholm V (1974). Students' Flora of Egypt. Cairo University. Cooperative Printing Company, Beirut, Lebanon, 888.

Temerk H, Salem W, Sayed W, Hassan FS (2017). Antibacterial effect of phytochemial extracts from *Ziziphus-spina christi* against some pathogenic bacteria. Egyptian Journal of Botany 57(3):595-604. *https://doi.org/10.21608/EJBO.2017.665.1035*

Trease GE, Evans WC (1989). Pharmacognosy. 13th Edition, Bailere Traiadal London, pp 69.

Tung YT, Chua MT, Wang SY, Chang ST (2008). Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. Bioresource Technology 99(9):3908-3913. *https://doi.org/10.1016/j.biortech.2007.07.050*

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5(3):93. *https://doi.org/10.3390/medicines5030093*

Üstün N, Bulam S, Peksen A (2018). Biochemical properties, biological activities and usage of truffles. International Congress on Engineering and Life Science, Kastamonu, Turkey 26-29. *https://www.researchgate.net/publication/328738891*

Villares A, García-Lafuente A, Guillamon E, Ramos Á (2012). Identification and quantification of ergosterol and phenolic compounds occurring in *Tuber* spp. truffles. Journal of food composition and analysis 26(1-2):177-182. *https://doi.org/10.1016/j.jfca.2011.12.003*

Wang S, Marcone MF (2011). The biochemistry and biological properties of the world's most expensive underground edible mushroom: truffles. Food Research International 44:2567-2581. *https://doi.org/10.1016/j.foodres.2011.06.008*

Wu Z, Meenu M, Xu B (2021). Nutritional value and antioxidant activity of Chinese black truffle (*Tuber indicum*) grown in different geographical regions in China. Lwt- Food Science and Technology 135:110226. *https://doi.org/10.1016/j.lwt.2020.110226*

Yadav RNS, Agarwala M (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology 3(12). *file:///C:/Users/WIN7/Downloads/1.Phytochemicalanalysisofsomemedicinalplants1.pdf*

Yan X, Wang Y, Sang X, Fan Li (2017). Nutritional value, chemical composition and antioxidant activity of three *Tuber* species from China. AMB Express 7:136-138. *https://doi.org/10.1186/s13568-017-0431-0*

Ziaei A, Ramezani M, Wright L, Paetz C, Schneider B, Amirghofran Z (2011). Identification of spathulenol in *Salvia mirzayanii* and the immunomodulatory effects. Phytotherapy Research 25(4):557-562. *https://doi.org/10.1002/ptr.3289*