

Gas Chromatography–Mass Spectrometry Analysis of Bioactive Compounds of Iraqi Truffle *Terfezia claveryi* (Ascomycetes), Synthesis of Silver Nanoparticles, and Appraisal of Its Biological Activities

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ABSTRACT: Microwave-assisted extraction (MAE) is a highly selective method that reduces the time and number of solvents used, has high efficiency, and is environmentally friendly. This study aimed to evaluate the antioxidant, anticancer, and antibacterial activities of Iraqi *Terfezia claveryi* desert truffle extract and silver nanoparticles by MAE using water and hexane as extraction solvents. Chemical compositions were identified by gas chromatography–mass spectrometry. Silver nanoparticles were characterized by field emission scanning electron microscopy. Antioxidant activity was assessed with the 2,2-diphenyl-1-picrylhydrazyl method. Antibacterial activity was evaluated against four types of pathogenic bacteria, and anticancer activity was also assessed. The *T. claveryi* hexane extract showed approximately 18 bioactive compounds, with an antioxidant half-maximal inhibitory concentration of 6.896 µg/mL. This extract also had significant antibacterial action against *Escherichia coli* and *Pseudomonas aeruginosa*. Cytotoxicity test of the hexane extract showed moderate toxicity against hepatocellular carcinoma mouse (HCA-M) liver cancer cells. Aerialine crystal ethidium bromide staining showed apoptosis and DNA damage in HCA-M cells treated with the hexane extract. The results of this study suggest that components of the *T. claveryi* wild desert truffle could have important nutrition functions that might support the immune system in fighting liver diseases when taken in daily regular doses.

KEY WORDS: *Terfezia claveryi*, liver cancer, silver nanoparticles, microwave-assisted extraction, medicinal mushrooms

ABBREVIATIONS: AgNP, silver nanoparticle; AO/EB, aerialine crystal ethidium bromide; DMSO, dimethylsulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GC-MS, gas chromatography–mass spectrometry; HCA-M, hepatocellular carcinoma mouse; IC₅₀, half-maximal inhibitory concentration; MAE, microwave-assisted extraction; MCF-7, Michigan Cancer Foundation-7; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NIST, National Institute of Standards and Technology; NP, nanoparticle; PC-3, human prostate cancer cell line; RL, retention time; SEM, scanning electron microscopy; T1w, *Terfezia claveryi* truffle hexane extract; T1w, *T. claveryi* truffle water extract

1. INTRODUCTION

Wild edible mushrooms contain healthy nutritional components that are influenced by environmental conditions. Many types of wild fungi have been identified as edible with low toxicity.^{1,2} The diversity and composition benefits of wild edible mushrooms represent a good food source for individuals living in rural areas.³ The chemical composition of mushrooms includes carbohydrates, proteins, and low levels of fatty acids, making them part of a healthy diet for humans.⁴ One such example is truffle (*Terfezia claveryi* Chaili (Tremellaceae, Ascomycetes)).⁵ The amount of truffles produced depends on the amount of rain and different environments of the various locations in which it grows, which can also affect its chemical composition.⁵

Several worldwide studies have shown important biological functions of the Iraqi truffle species of genus *Terfezia*,^{6,7} such as antibacterial,⁸ anticancer,⁹ and antioxidant functions.¹⁰ *T. claveryi* also has significant levels of nutrients, such as proteins, carbohydrates, and fats. Extraction methods using various solvents can