Antioxidant activity of methanolic extract from the fruiting bodies of Truffle species

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Abstract :

Truffles, the symbiotic hypogeous edible fungi, have been worldwide regarded as a great delicacy because of their unique flavor and high nutritional value. By identifying their bioactive components such as phenolics, terpenoids, polysaccharides, fatty acids, and ergosterols, researchers have paid attention to their biological activities including antitumor, antioxidant, antibacterial and anti-inflammatory activities. In current study methanol extracts of truffle were used to assess qualitative chemical assessment and antioxidant activity . The results showed the methanol truffle extract has Phenolic and alkaloids compounds and has antioxidant activity which showed non-significant differences ($P \leq 0.05$) for two high concentration 1000 and 2000 µg/ml as compared with all standard solution concentration (vitamin C). Also result showed non-significant differences under the same probability level in the values of the IC_{50} between all truffle extract concentration and vitamin C .

Introduction:

Truffles are hypogenous ascomycetes fungi growing underground in depth between 5 and 10 cm, are referred to a “underground gold” because they are rare and are highly valued for their culinary and medicinal traits (Tang *et al*., 2015). More than a hundred different kinds of truffle species were known worldwide, and new species are being discovered continuously.

Truffle are ectomycorrhizal having symbiotic root association. In a complex life cycle, the mycelia establish a symbiotic interaction with host organisms predominantly with the roots of various trees. Taxonomically, edible truffles belong to family Tuberaceae and Pezizaceae, and to order Pezizales (Dahham *et al*., 2018).

Figure (1): Truffle fruiting body

Truffle is one of the oldest forms of food, it has been used as a meat substitute and consumed in large quantities due to their highly delicious taste and musky aroma (Dundar *et al*., 2012). It has a unique nutritional profile of unsaturated fatty acid, vitamins, minerals, and protein (Patel, 2012) and has been used for eye treatment in folk medicine (Janakat and Nassar, 2010). *Terfezia claveryi* is among the

various known edible truffles in the world, including Iraq where it grows naturally in the central, southern and western parts.

The nutrients and bioactive compounds with health benefits in truffles are also the major attraction, leading to a series of investigations to explore the metabolites in their fruiting bodies. It has been found that truffles are rich in protein, fatty acids, carbohydrates, amino acids, and minerals (El Enshasy *et al*.,2013 ; Lee *et al*., 2020).

Truffles are rich in various types of essential nutritional products including carbohydrates, proteins, fats, minerals, lipids, and amino acids. In addition, they are rich in phenolic, terpenoids, polysaccharides, and phytosterols which are related to their antitumor, antioxidant antibacterial, hepatoprotective, anti-infammatory, and immunomodulatory properties (Om et al., 2021). Figure (2).

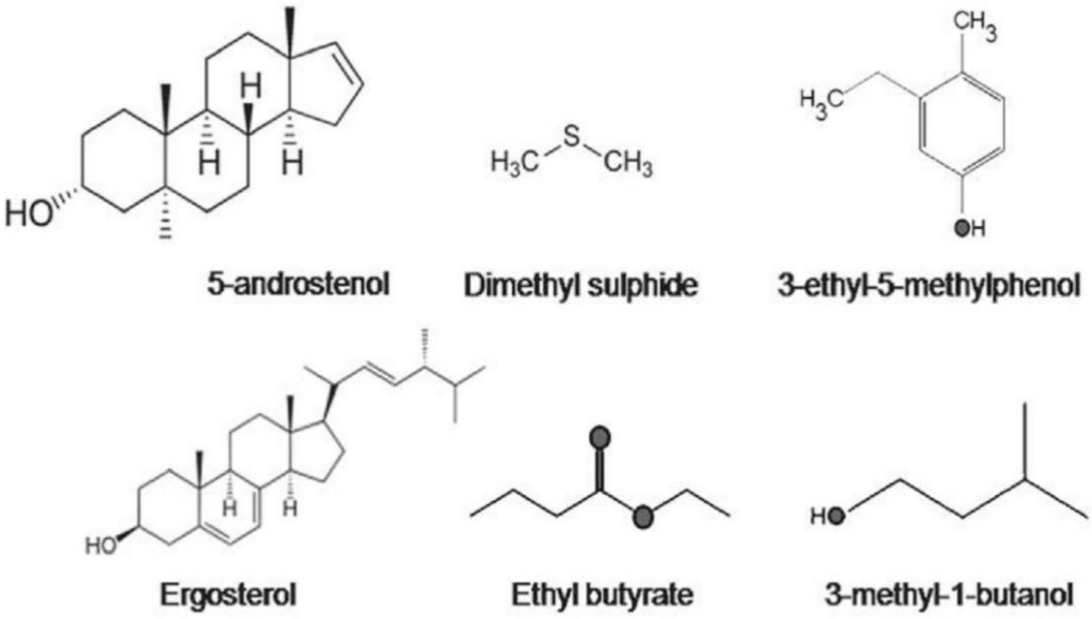


Figure (2) : Some aromatic compounds of truffles

In recent decades, researchers have paid great attention to the bioactive compounds derived from diverse truffle species and their potential in nutritional and medicinal applications (Om *et al*., 2021).

Figure (3) : Truffles products available in the market

Materials and methods

Extraction

Truffles were purchased from market. Fresh truffles were cleaned well (by brush followed by wet towel), sliced to the thickness of about 1–3 mm, dried, powdered and kept in dry and dark place. The dried powder (250 mg) were extracted with methanol 80 % at room temperature for 3 days. Then the extract was filtered and the supernatant was concentrated and drying , figure (4) (Maha *et al*., 2020).

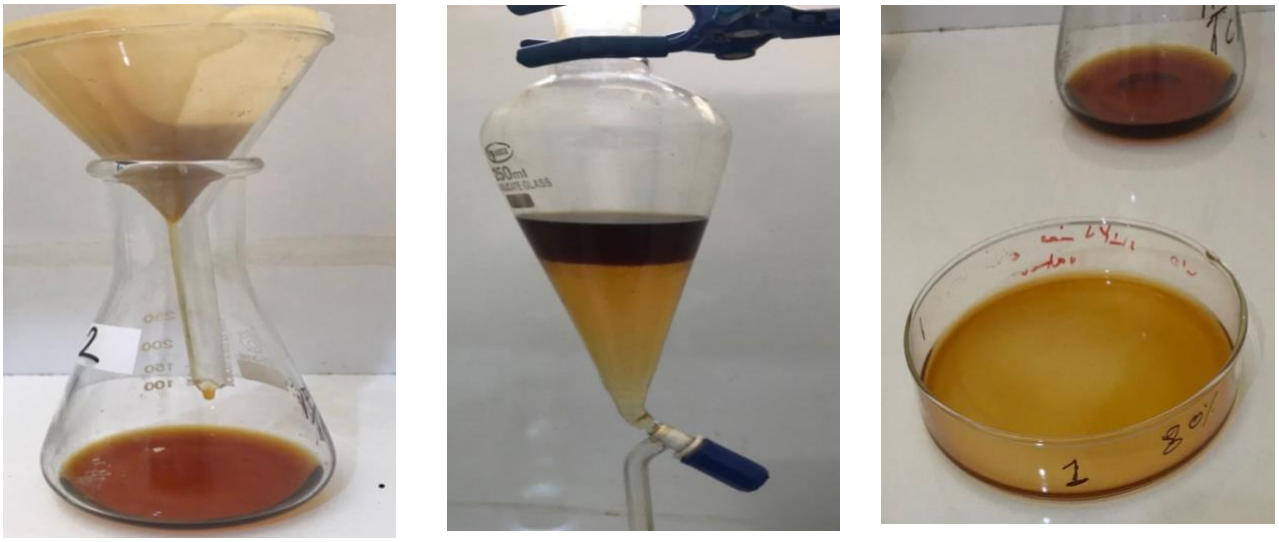


Figure (4): Extract steps of truffle

Qualitative chemical assessment

Several different chemical analyses were carried out on the extracts to identify their primary components (Thilagavathi *et al* ., 2015). The following procedures were followed during the tests:

1- **flavonoids test :** Using Shinoda test: A few fragments of magnesium ribbon and concentrated hydrochloric acid were added to the methanolic extract. The appearance of red to pink color after few minutes indicates the presence of flavonoids.

2- **Phenols test :**Using ferric chloride test: A few drops of neutral ferric chloride solution were added to the methanolic extract. Formation of green, blackish green color indicates the presence of phenolic compounds.

3- **Alkaloids test :**Using Dragendroff's reagent: equivalent amount of the reagent were added to the methanolic extract. The appearance of an orange precipitate indicated the presence of alkaloids.

Antioxidant activity

The antioxidant activity of the truffle extract was determined by the DPPH free radical scavenging assay in triplicate. According to (Yen *et al*., 2002), a freshly prepared (0.004% w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). 0.5 ml of this solution was added to 2.5ml of three different concentrations of the truffle extract (2000., 1000 and 500 µg/ml) were prepared in methanol 80% and same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant and was allowed to stand at room temperature for 30 min. and then absorbance was measured with a UV–visible spectrophotometer at 517 nm against blank sample.

Inhibition of free radical DPPH in percent (I %) was calculated in following way:

I %= (Blank– Sample / Blank) x 100

where blank is the absorbance of the control reaction (containing all reagents except the test compound), and sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration.

The Results

Phytochemical assay

Phytochemical assay for truffle extract showed negative test for flavonoids, while positive test for phenols and alkaloids. Figure (5) .

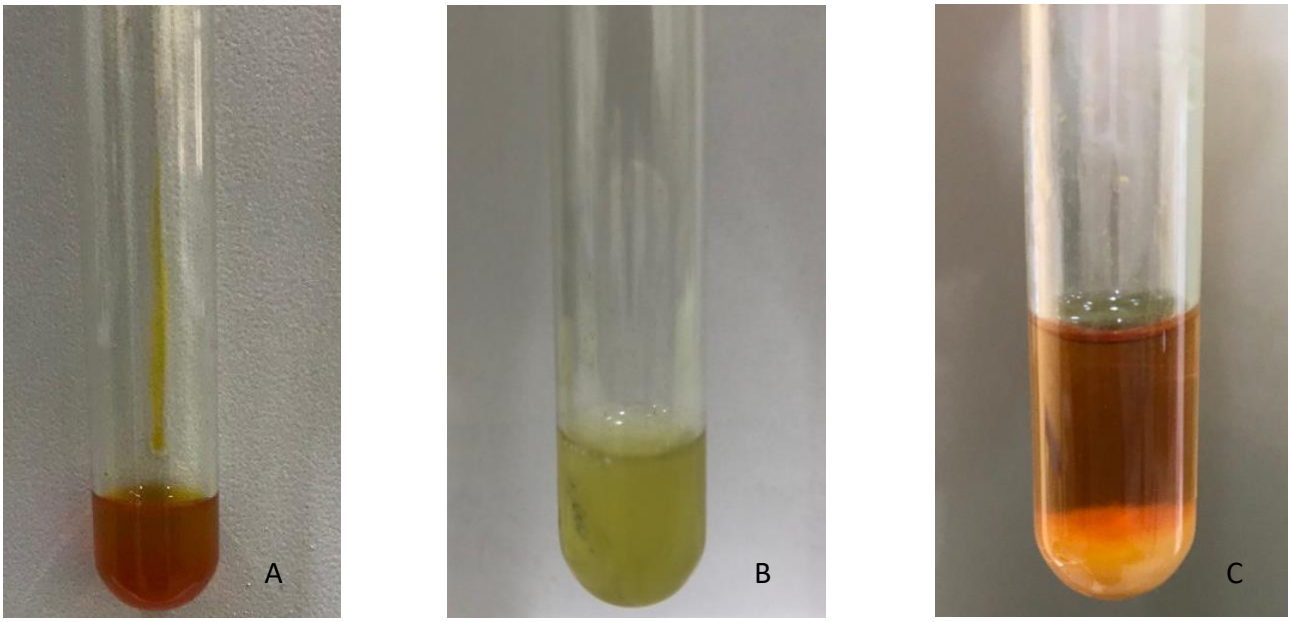
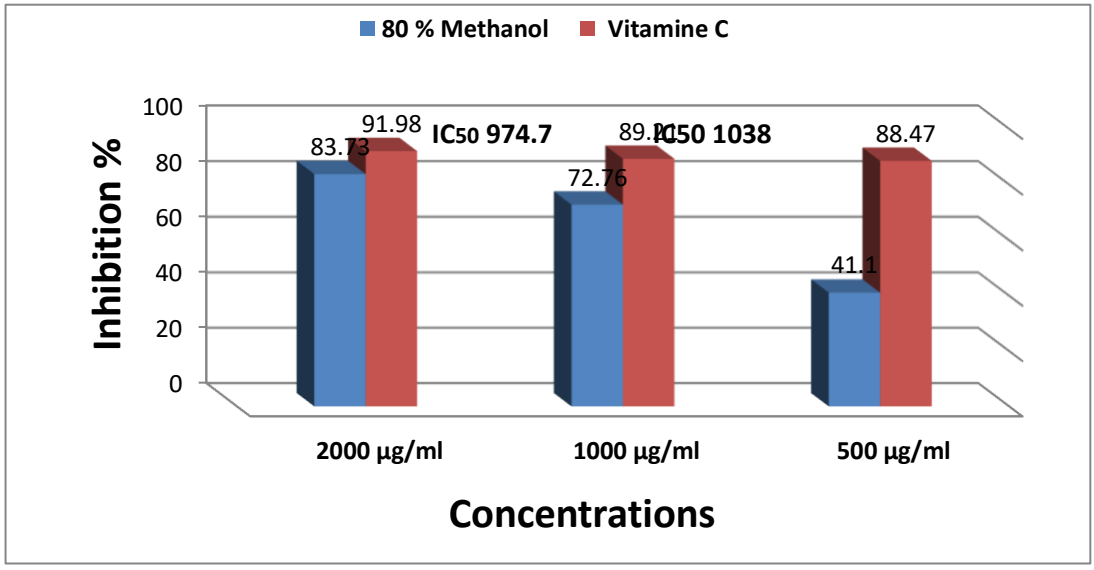


Figure (5) : Phytochemical assay A: Flavonoids. B: Phenols. C: Alkaloids

The phytochemical test showed positive results for phenols in aqueous methanol extract. This result agrees with Maha *et al*.,(2020) . Previous study confirm the potential activity of phenols in decreasing the risk of developing many types of cancer and decreasing the risk of cancer progression, due to their ability to decrease vascular endothelial growth factor (VEGF) expression and cell viability (He *et al*., 2015).

Antioxidant activity

Finding showed that the concentrations 1000 and 2000 µg / ml of truffle extract did not show any significant differences with the control group (vitamin C) in its oxidative activity, except for the concentration 500 µg / ml, which showed significant differences, where the effectiveness reached 41.1% compared to vitamin C , which had the highest effectiveness of 88.47% figure (6). Also results was showed non-significant difference between the IC_{50} for truffles extracts and Vitamin C which reach to 974.7 and 1038 µg / ml respectively



Figure(6): Antioxidant activity percentage of Truffle extracts under three different concentrations compared with vitamin C

The antioxidants play an important role in diminishing the harmful effects of ROS that correlated damage to cell membranes , enzymes and DNA molecules. Oxidative stress in a crucial factor that contributes to the high mortality rates associated with dysregulation of the immune system , which in turn leads to a number of diseases (Valko *et al.*, 2007) .

Antioxidants may provide protection extracellular by scavenging ROS and intracellular by preventing lipid peroxidation inside the membrane. Other publications suggested that phenolic compounds were those responsible for antioxidant activities, such as in methanol extracts obtained from *T. magnatum* Some important metabolites with biological activities have been isolated and characterized from truffle fruiting bodies, including polysaccharides, ergosterol, and phenolics, which have been verified to possess multiple healthy attributes (Patel et al.,2017), this compounds of truffle fruiting bodies showed high antioxidant potential and antitumor activities. In addition, anti-inflammation. Many of the alkaloids had (.Al Obaydi , *et al.*, 2020).

Conclusion :

Our present study concludes that truffle extract possess antioxidant activity properties which could be due to presence of active constituents presence in the truffle extract such as phenol and alkaloid.

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