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Antioxidant and antigenotoxic potential of *Ramaria largentii* Marr & D. E. Stuntz, a wild edible mushroom collected from Northeast Romania

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ABSTRACT

Ramaria largentii Marr & D. E. Stuntz (orange coral mushroom) is a wild edible mushroom whose chemical composition and bioactivity have not been investigated. Herein, we present a study on the phenolic constituents, antioxidant and antigenotoxic effects of a hydromethanolic extract of the fruiting bodies. Total phenolic content, estimated by Folin-Ciocalteu assay, was found to be 42.33 ± 0.18 mg GAE/g. Protocatechuic and vanillic acids were detected by HPLC-DAD-ESI-MS. The extract showed good free radical scavenging and reducing capacities (EC_{50} = 64.3 ± 0.2 and 61.54 ± 0.46 μg/mL, respectively). In normal Vero cells, the extract (100, 200 and 300 μg/mL) showed no genotoxic potential and moreover, almost completely protected DNA against H_2O_2-induced damage (2.09–7.91% tail DNA) (24 and 48 h pre-treatment). Taken together, the results of our study show that *Ramaria largentii* extract is devoid of genotoxicity and has a remarkable DNA protective activity against H_2O_2-induced damage.

1. Introduction

From ancient times edible mushrooms have been used both as food and medicine. Besides their attractive taste and aroma, edible mushrooms have an important nutritional value since they are rich in proteins (15.2–80.93% dry weight), carbohydrates (35–70% dry weight), vitamins (B_1, B_2, B_{12}, C, D, E, β-carotene), minerals (6–10.5% dry weight, mainly K, P, Mg, Ca, Cu, Fe, Zn) and low in fats. Edible mushrooms are a good source of essential amino acids (arginine, aspartic and glutamic acids) and dietary fibers (β-glucans, chitin). With regard to the fat content, palmitic, oleic and linoleic acids are the most abundant. Edible mushrooms also contain bioactive secondary metabolites such as phenolic compounds, sterols and triterpenes (Guillamón et al., 2010). These nutrients and metabolites are responsible for the biological activities reported for edible mushrooms (antioxidant, anti-inflammatory, hypoglycemic, hypocholesterolemic, antihypertensive, antitumor, immunomodulatory, hepatoprotective, antibiotic and antiviral effects) (Boa, 2004; Cheung, 2010; Guillamón et al., 2010).

Oxidative stress is involved in aging but also in pathological conditions such as cancer, cardiovascular disease, ischemic/reperfusion injury, rheumatoid arthritis, diabetes and neurological disorders (Valko et al., 2007). There is a consistent body of evidence from epidemiological and interventional human trials that antioxidant-rich foods and antioxidant supplementation reduce the risk and incidence of many chronic diseases (Ferreira et al., 2009). The antioxidant potential of edible mushrooms has been extensively investigated in both *in vitro* and *in vivo* models. Various extracts of edible mushrooms were found to scavenge reactive oxygen species (superoxide anion radical, hydroxyl radical, H_2O_2) and nitric oxide, chelate pro-oxidant transition metal ions, reduce lipid peroxidation, increase the levels of non-enzymatic antioxidants (vitamins C and E, glutathione), stimulate antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase,

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glutathione reductase) and inhibit xanthine oxidase (Ferreira et al., 2009; Jia et al., 2009; Kozarski et al., 2015).

DNA oxidation leads to gene mutations and chromosomal aberrations which play an important role in the development of cancer and other severe disorders (neuromuscular, neurodegenerative, cardiovascular, metabolic and immune disorders) (Jackson and Bartek, 2009). Human body is permanently exposed to genotoxic agents that cause DNA oxidation (UV light, nicotine in tobacco smoke, pollutants in water and foods such as aflatoxin B1 and ochratoxin A, food processing by-products such as benzo(a)pyrene and nitrosamines). Oxidative stress-induced DNA damage might also arise during anticancer chemotherapy and radiotherapy (Jackson and Bartek, 2009; Luca et al., 2016). Therefore, identification of new potential antigenotoxic agents, efficient and safe, is of great interest. The ability of several edible mushroom extracts but also mushroom constituents to protect DNA against genotoxic agents has been investigated in different cell lines (Burkitt's lymphoma cells, Chinese hamster lung fibroblastic V79 cells, human laryngeal epidermoid carcinoma HEp2 cells, human hepatoma HepG2 cells, human lymphocytes). Extracts of *Agaricus bisporus*, *Ganoderma lucidum*, *Inonotus obliquus, Agrocybe cylindracea, Agaricus blazei*, *Lentinula edodes* (shiitake), *Lactarius vellereus* afforded DNA protection in cells exposed to different genotoxicants such as H_2O_2, hydroxyl radicals (Roupas et al., 2012; Shi et al., 2002b), methyl methanesulfonate (Guterres et al., 2005; Miyaji et al., 2004), cyclophosphamide (Delmanto et al., 2001) and 2-amino-3-methylimidazo(4,5-f)quinoline (Mlinarič et al., 2004).

The genus *Ramaria* comprises approx. 300 species distributed worldwide; some species are edible whereas others (*R. formosa*, *R. pallida*) are mildly poisonous causing nausea, vomiting and diarrhea (Łuszczyński, 2009; Nasim et al., 2008). Edible mushrooms of the genus *Ramaria* have been poorly investigated. Few studies reported only on the constituents and biological activities of *R. aurea* (Khatua et al., 2015), *R. flava* (Gursoy et al., 2010; Liu et al., 2013) and *R. botrytis* (Barros et al., 2009). *R. largentii* (orange coral mushroom) is an edible species usually living in habitats with conifers (Ouzouni et al., 2009). No studies have investigated the chemical constituents and biological effects of this species. Only the heavy metal accumulation in the fruiting bodies was reported (Ouzouni et al., 2009; Rieder et al., 2011). In the present work, a hydromethanolic extract of the fruiting bodies was investigated regarding its phenolic profile, antioxidant and antigenotoxic potential.

2. Material and methods

2.1. Chemicals

Gallic, caffeic, protocatechuic and vanillic acids, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium ferricyanide, ferric chloride, sodium carbonate, sodium hydroxide, linoleic acid, formic acid, dimethyl sulfoxide (DMSO), sodium lauryl sarcosinate, normal and low melting point agarose were purchased from Sigma-Aldrich (Steinheim, Germany). Ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (ferrozine), H_2O_2 and lipoxidase from soybean were supplied by Fluka (Steinheim, Germany). Folin-Ciocalteu's phenol reagent, ferrous chloride and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were from Merck (Darmstadt, Germany). Ethidium bromide was obtained from Carl Roth (Karlsruhe, Germany). Trichloroacetic acid and potassium persulfate were from Riedel-de Haën (Seelze, Germany). Dulbecco's Modified Eagle's medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum, streptomycin and penicillin were from Biochrom AG (Berlin, Germany). All other solvents and reagents were of analytical grade. Ultrapure water was obtained using an Ultra Pure Water System type Ultra Clear TWF UV (SG Water, Barsbüttel, Germany).

2.2. Mushroom species

Fruiting bodies of *R. largentii* Marr & D. E. Stuntz (Gomphaceae) were collected in Poiana Stampei (Northeast of Romania) in September 2013. Authentication was made at the Laboratory of Mycology and Phytopathology, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Romania. Voucher specimens were deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Grigore T. Popa University of Medicine and Pharmacy Iasi, Romania.

The fruiting bodies were cleaned without washing, freeze-dried and powdered.

2.3. Extraction

Dried and powdered fruiting bodies (50 g) were extracted with 96% ethanol as previously described (Zavastin et al., 2015b). The residue was further extracted twice, each time with 500 mL of methanol:water (1:1, v/v) under stirring at 350 rpm for 3 h at room temperature in dark condition. The hydromethanolic extracts were pooled together, evaporated to dryness under reduced pressure at 40 °C and stored at −18 °C.

2.4. Estimation of total phenolic content

Total phenolic content was estimated by Folin-Ciocalteu assay (Wangensteen et al., 2004). In brief, the extract (25 mg/mL, 40 μL) was mixed with ultrapure water (3.16 mL) and Folin-Ciocalteu reagent (200 μL). After 5 min, 20% sodium carbonate (600 μL) was added. The reaction mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm. The phenolic content was expressed as mg of gallic acid equivalents (GAE)/g of extract.

2.5. RP-HPLC-DAD-ESI-MS analysis of phenolic compounds

The analysis of phenolic compounds was performed by reversed-phase high performance liquid chromatography (RP-HPLC) using an Agilent 1200 Series HPLC system with diode array detector (DAD) coupled to an Agilent 6520 accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with an electrospray ionization (ESI) source. The separation was carried out on a 250 × 4.6 mm, 5 μm Hypersil ODS C18 column (Thermo Scientific). The mobile phase consisted of acetonitrile (A) and water with 0.1% formic acid (B). A gradient elution varying from 0% to 100% A over 90 min was applied. The injection volume was 20 μL. The flow rate and detection wavelength were set to 0.5 mL/min and 280 nm, respectively. The mass spectrometric detection was performed in the negative ion mode (capillary voltage −4.0 kV, skimmer voltage −68 V, drying gas flow rate 7 L/min, drying gas temperature 235 °C, nebulizer pressure 25 psig). Masses were scanned from 80 to 2800 amu in steps of 0.3 amu. MassHunter Workstation software was used for data processing. Phenolic compounds were identified by comparing their retention times, UV and ESI-MS spectra with those of authentic standards.

2.6. ABTS radical cation scavenging assay

ABTS radical cation was generated according to Re et al. (1999) by incubating ABTS (7 mM) with potassium persulfate (2.45 mM) for 12–16 h in dark followed by dilution with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm (equilibration at 30 °C).