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Nutritional value and antibacterial activity of desert truffle *Tirmania pinoyi* (Maire) Malençon (1973) growing wild in Libya

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Introduction

Mushrooms are widely appreciated for their nutraceutical value, since edible species are widely consumed all over the world. They have a long history of use for their health promotion benefits (Zaidman et al., 2005). Fungal species are considered to be a good source of many different compounds belonging to unsaturated fatty acids, phenolic compounds, tocopherols, proteins, and other bioactive metabolites (Barros et al. 2009). In recent years reports on the chemistry, and the nutritional and functional properties of mushroom have been overwhelming and more than 300 articles related to mushrooms have been published in the last two decades (Rathee et al., 2012).The golden age of antibiotic discovery is the 20th century and these potent drugs indisputably saved millions of lives. Nevertheless, we are entering an era when bacterial infections might no longer be successfully treated with commercially available antibiotics; overuse of antimicrobial drugs resulted in the emergence and spreading of multidrug and even pan-resistant pathogenic microorganisms (Carlet et al., 2011), so the search for new antimicrobial agents must be continued. Antimicrobial potential of mushrooms is still poorly investigated and available literature (Ofodile et al., 2011; Bala et al., 2011) indicates that further chemical analyzes of mushrooms is required to support studies searching for mushroom derived antimicrobial agents.

Desert truffles, though less appreciated in cuisine than the European forest ones, are nonetheless true truffles in the sense that they are hypogeous (underground) ascomycete fungi, as opposed to hypogeous basidiomycete fungi, termed false truffles. The best known and appreciated genera *Terfezia* and *Tirmania* were shown to belong to the Pezizaceae rather than to the distinctly hypogeous Terfeziaceae family, which has been abolished (Norman and Egger, 1999).

The main goals of our study were to investigate nutraceutical value, sugars and fatty acids composition of *Tirmania pinoyi,* as well as to determine antibacterial properties of its methanol extract dissolved in three different solvent systems.

Materials and Methods

Mushroom species. Tirmania pinoyi (Maire) Malençon (1973) was collected during July of 2011 in Libya and authenticated by Dr. Jasmina Glamočlija and Dr. Marina Soković. A voucher specimen has been deposited at the Fungal Collection Unit of the Mycological Laboratory, Department for Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, under number TpAG-2011.

Nutritional value. The samples were analysed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content (N×4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

Sugar composition. Free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RI detector Knauer Smartline 2300) as previously described by the authors (Reis et al., 2011). Sugar identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

Fatty acid composition. Fatty acids were determined after a transesterification procedure as described previously by the authors (Reis et al., 2011), using a gas chromatographer (DANI 1000) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7).

Preparation of the extract. Powdered sample of *T. pinoyi* (20 g) with 0.1M enzyme inhibitor (peroxidases and oxidases) potassium metabisulphite was extracted by stirring with 400 mL of methanol (25°C) for 72h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with 200 mL of methanol (25°C at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40°C (rotary evaporator Büchi R-210) to dryness. The extract was redissolved in i) methanol:water (30:70) ii) sterilized distilled water containing 0.02% Tween 80 iii) 5% DMSO for antimicrobial activity assay.

Antibacterial activity. For the bioassays eight bacterial strains were used: *Staphylococcus aureus* (food isolate), *Micrococcus flavus* (ATCC 10240), and *Listeria monocytogenes* (NCTC 7973), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (food isolate), *Escherichia coli* (ATCC 35210), *Salmonella enteritidis* (ATCC 13076), and *Salmonella typhimurium* (ATCC 13311).

In order to investigate the antimicrobial activity of the extract, the modified microdilution technique was used (NCCLS, 1999). Bacterial species were cultured overnight at 37 °C in Tryptic Soy Broth (TSB). The bacterial cell suspensions were adjusted with sterile saline to a concentration of approximately 1.0×10^6 in a final volume of 100 μL per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid (Müller-Hinton) MH for bacteria to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentrations (MICs) were determined by a serial dilution technique using 96-well microtitre plates. The samples tested were dissolved in 5% solution of DMSO (10 mg/mL) and added to TSB with inoculum. The microplates were incubated for 48 h at 37 °C. The lowest concentrations without visible growth (using a binocular microscope) were defined as MICs. The minimum bactericidal concentrations (MBCs) were determined by serial subcultivation of 2 μL into microtitre plates containing 100 μL of TSB per well and further incubation for 48 h at 37 °C. The lowest concentration with no visible growth was defined as MBC, indicating a 99.5% kill of the original inoculum.

Streptomycin (Sigma P 7794), (0.05-3 mg/mL) was used as a positive control for bacterial growth. Methanol:water (30:70), sterilized distilled water containing 0.02% Tween, 80 and 5% DMSO were used as negative controls as well as potassium metabisulphite. All experiments were done in triplicate and repeated twice.

Results and Discussion

Tirmania pinoyi nutraceutical value was evaluated. The results showed the following content: Ash – 5.26 g/100 g dw; Proteins – 8.06 g/100 g dw; Fat – 4.09 g/100 g dw; Carbohydrates – 82.6 g/100 g dw; Energy – 399.4 kcal/100 g dw. As for the sugar analysis, we found the following free sugars present in *T. pinoyi*: Rhamnose – 1.72 g/100 g dw, Fructose – 0.13 g/100 g dw, Trehalose – 1.93 g/100 g dw and Mannitol – 0.31 g/100 g dw. HPLC analysis of fatty acids revealed the presence of 23 fatty acids: C6:0 (Caproic acid) – 1.89; C8:0 (Caprylic acid); C10:0 (Capric acid); C12:0 (Lauric acid); C14:0 (Myristic acid); C15:0 (Pentadecanoic acid); C16:0 (Palmitic acid); C16:1 (Palmitoleic acid); C17:0 (Heptadecanoic acid); C18:0 (Stearic acid); C18:1n9c (Oleic acid); C18:2n6c (Linoleic acid); C18:3n3 (Linolenic acid); C20:0 (Arachidic acid); C20:1 (cis-11-Eicosenoic acid); C20:2 (cis-11,14-Eicosadienoic acid); C20:3n3+C21:0 (cis-11,14,17-Eicosatrienoic acid and Heneicosanoic acid); C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic acid); C22:0 (Behenic acid); C22:1n9 (Erucic acid); C23:0 (Tricosanoic acid); C24:0 (Lignoceric acid). The fungus examined contained 35.82 % of total saturated fatty acids, 33.50% of total monounsaturated fatty acids and 30.67 % of total polyunsaturated fatty acids.

Results of antimicrobial activity are presented in the Table listed below.

Table. Antibacterial activity of *T. pinoyi* evaporated methanol extract and dissolved in three different solvent systems (mg/mL).

Bacteria	water tween		30:70% methanol:water		5% DMSO	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	3.00	3.00	3.00	3.00	3.00	3.00
S. enteritidis	3.00	3.00	3.00	3.00	3.00	3.00
P. aeruginosa	3.00	3.00	3.00	3.00	3.00	3.00
E. coli	1.25	2.50	0.62	1.25	1.25	2.50
En. cloacae	3.00	3.00	3.00	3.00	3.00	3.00
S. typhimurium	3.00	3.00	3.00	3.00	3.00	3.00
L. monocytogenes	1.25	2.50	1.25	2.50	1.25	2.50
M. flavus	1.25	2.50	0.62	1.25	1.25	2.50

It is evident from the above table that the extract possessed an antibacterial effect. The best solvent system for *in vitro* study seemed to be methanol:water (30:70). Extract dissolved in this system had a better antimicrobial effect on *E. coli* and *M. flavus* in correlation to the other solvents used to dilute the evaporated methanol extract (water tween and 5% DMSO). The best results were achieved for the same mentioned species with MIC of 0.62 mg/mL and MBC of 1.25 mg/mL. All of the results obtained were comparable to those obtained previously for standard antibiotics.

Conclusions

- Since *T. pinoyi* is very good source of potent antimicrobial compounds, further analyses are required for determination and chemical characterization of antibiotics from *T. pinoyi.*
- We suggest the use of *T. pinoyi* as spice for preservation of food from microbial contamination, since it has good inhibitory activity against food spoilage bacteria and high nutritional value.

References

1. AOAC. (1995). Official methods of analysis (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.
2. Bala N, Aitken EA, Fechner N, Cusack A, Steadman KJ. *Pharm Biol.* 2011, **49**: 492-500.
3. Barros L., Dueñas M., Ferreira I.C.F.R., Baptista P., Santos-Buelga C. *Food Chem Toxicol* 2009, **47**: 1076-1079.
4. Carlet J, Collignon P, Goldmann D, Goosens H, Gyssens IC, Harbarth S, Jarlier V, Levy SB, N'Doye B, Pittet D, Richtmann R, Seto WH, van der Meer JW, Voss A. *Lancet* 2011, **378:** 369-371.
5. NCCLS (National Committee for Clinical Laboratory Standards) (1999). Performance standards for antimicrobial susceptibility testing; 9th International Supplement, Wayne Pa. M100-S9.
6. Norman JE, Egger KN. *Mycologia* 1999, **91**: 820-829.
7. Ofodile LN, Uma N, Grayer RJ, Ogundipe OT, Simmonds MS. *Phytother Res.* 2011, doi: 10.1002/ptr.3598.
8. Rathee S, Rathee D, Rathee D, Kumar D, Rathee P. *Rev. bras. farmacogn.* 2012, **22**:459-474.
9. Reis FS, Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, Ferreira, ICFR. *J Food Sci* 2011, **76**: 824-830.
10. Zaidman BZ, Yassin M, Mahajna J, Wasser SP. *Appl Microbiol Biot* 2005, **67**: 453-468.