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# Biological potential of puffballs: A comparative analysis

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ABSTRACT

*Handkea utriformis* (HU), *H. excipuliformis* (HE) and *Vascellum pratense* (VP) mature fruiting bodies methanol extracts were tested for biological activities and active compounds. The extracts showed prominent radical scavenging, reducing, antioxidative and chelating abilities. Scavenging ability was correlated with phenolic (ABTS assay) or phenolic and/or sugar/β-glucan content (DPPH). Antioxidative and $Fe^{3+}$-reducing ability of VP extract was the highest, and was best correlated with flavonoid content. The same extract exhibited the best angiotensin-converting enzyme inhibitory activity. HU and HE showed selectivity toward tumor cell lines in cytotoxicity analysis. The extracts exhibited various antimicrobial activity, the best being against *Listeria monocytogenes* (HE, MIC-0.625 mg/mL); fatty acid content was particularly high in HU (37.25 mg/g), with linoleic acid making up more than 57% in all samples. Phenolics were present in considerable amount, as well as β-glucans (HU, 16.67%). Although these mushrooms are inedible after autolysis process, they were still a good source of biologically active products.



## 1. Introduction

The puffballs are fungi that produce enclosed fruiting bodies – mushrooms – with white gleba that turns into a brown powdery mass of spores upon maturation. The species are mostly saprotrophs (Larsson & Jeppson, 2008), though some species are reported to engage mycorhyzes (Coetzee & van Wyk, 2009), and are growing in the forest soil, on the rotting wood or in the grasslands. There are about 150 described species of

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puffballs, which are widely spread around the world (Larsson & Jeppson, 2008).

Most puffball species are edible while their fruiting bodies are young, before the spore development. Species of *Calvatia*, *Handkea* (earlier included in genus *Calvatia*), *Lycoperdon* and *Vascellum* are used for human consumption in some areas. In general, puffballs have long been respected for their culinary value (Læssøe & Spooner, 1994) and species such as *Calvatia gigantea* and *Handkea utriformis* have been rated particularly high due to their organoleptic properties (Coetzee & van Wyk, 2009).

Uses of puffballs in traditional medicine are many and widespread. Though they had long history, biologically active compounds of some species have been isolated and characterized only relatively recently. The most common use of puffballs is probably as a wound dressing, since mature, dry gleba of most species is useful and effective styptic. This feature was widely appreciated in Europe, North America, Africa (Nigeria) and Asia (India and China). In China, many gasteroid fungi, including puffballs such as *Bovista*, *Bovistella*, *Calvatia*, *Lycoperdon* and *Mycenastrum* species have been considered as medically important, their usage being more diverse. Puffball species were believed to have detoxification properties and could reduce swelling, act against throat ailments, cough and fever, as a painkiller or repressing stomach ache (Læssøe & Spooner, 1994).

The traditional use of these mushrooms, both in culinary practice and folk medicine, leads to various research on their nutritional value, pharmacological and biotechnological potential (Coetzee & van Wyk, 2009), but still many species of puffballs have not yet been assessed for their potential benefits for humans. Though research on puffballs themselves is limited, numerous scientific reports have shown that mushrooms and their products have significant bioactive properties such as immunomodulation, anti-cancer, antioxidant, blood-pressure, cholesterol and sugar lowering, antimicrobial and antiviral etc (Mallavadhani et al., 2006; Roupas, Keogh, Noakes, Margetts, & Taylor, 2012; Santoyo, Ramírez-Anguiano, Reglero, & Soler-Rivas, 2009; Smolskaitė, Venskutonis, & Talou, 2015).

The objective of this study was to evaluate and compare antioxidant, ACE-inhibitory, antimicrobial and cytotoxic activity of three puffball species, *Handkea utriformis* (mosaic puffball), *Handkea excipuliformis* (pestle puffball) and *Vascellum pratense*. Though mature specimens with powdery gleba are no longer edible, it is estimated that they can still be an excellent sources of biologically active components.

## 2. Materials and methods

### 2.1. Sample collection

Three species of puffballs were used in this research, two species belonging to genus *Handkea*, namely *Handkea utriformis* (HU) and *Handkea excipulliformis* (HE), and *Vascellum pratense* (VP). For this investigation, only gleba (inner spore bearing mass) of the completely mature and dry specimens was used, excluding exoperidium. Fruiting bodies were collected in grassy areas near Bor, eastern Serbia (HU and VP) and Belgrade (HE).

### 2.2. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2, 6-dichlorophenolindophenol (DCPIP), aluminum chloride, ferrous sulfate, ferrous chloride, ferric chloride, potassium ferricyanide, potassium bromide, sodium carbonate, sodium nitrite, sodium hydroxide, sodium hydrogen carbonate, potassium persulfate, sulfuric acid, metaphosphoric acid, trifluoroacetic acid (TFA), linoleic acid, Tween 20, Ferrozine, phenol, triphenyltetrazolium chloride (TTC), dimethyl sulfoxide (DMSO), Folin–Ciocalteu reagent, RPMI-1640, fetal bovine serum (FBS), Hepes, l-glutamine, streptomycin, penicillin, amoxicillin trihydrate, fluconazole, cisplatin, angiotensin converting enzyme from rabbit lung (ACE) [EC 3.4.15.1], hippuryl-L-histidyl-L-leucine (HHL), standards such as ascorbic acid, ethylenediaminetetraacetic acid (EDTA), gallic acid, catechin and Trolox were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Co. (Darmstadt, Germany). Solvents such as methanol, ethanol, n-hexane and acetone were provided by LGC Promochem, Germany. Phytohaemaglutinin (PHA) was purchased from (Wellcome Diagnostics, Dartford, UK). The analytical mushroom β-glucan kit was obtained from Megazyme Int. (Wicklow, Ireland). Mediums necessary for microbial cultivation were purchased from Biolife (Milan, Italy). Enalpril maleate standard was provided by Salutas Pharma GmbH (Barleben, Germany). All other chemicals and reagents were either extra pure or of analytical reagent grade.

### 2.3. Extract preparation

Gleba of the mature specimens was undergone to maceration with absolute methanol for 72 h, with constant stirring. The mixture was then filtered and solvent evaporated under low pressure, at 30 °C (Heidolph Hei-VAP Value rotary evaporator, Schwabach, Germany). The extracts were additionally dried in vacuum dessicator at room temperature and kept in the refrigerator for later use. Drug:extract ratio (DER) was 6.2:1 for HU, 6:1 for HE and 5.9:1 for VP, very similar and low in all three cases (meaning higher extraction yield).

### 2.4. Determination of antioxidants, polysaccharides and free fatty acids

#### 2.4.1. Determination of antioxidants

Total phenolic compounds in the mushroom extracts were estimated by a colorimetric assay, based on procedure given by Skotti, Anastasaki, Kanellou, Polissiou, and Tarantilis (2014). Each extract methanol solution (0.1 mL, 20 mg/mL) was mixed with 0.5 mL of Folin and Ciocalteu's reagent and 6 mL of Milli-Q water. After 6 min, 1.5 mL of saturated $Na_2CO_3$ solution was added and the mixtures were adjusted to 10 mL with Milli-Q water. The reaction was kept in the dark for 2 h and the absorbance was read at 765 nm against the blank (solution of all reagents excluding extract). Gallic acid was used to calculate the standard curve (0.1–0.7 mg/mL; $y = 0.001x - 0.0214$; $R^2 = 0.9995$) and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Total flavonoids in the extracts were determined by a colorimetric assay described by Barros, Baptista, and Ferreira (2007).