ORIGINAL ARTICLE

QUALITATIVE AND QUANTITATIVE CHEMICAL STUDY OF *RUSSULA VIRESCENS* MUSHROOM

MARIA LIDIA POPESCU[1]*, MIHAELA CULMEŞ[2], CERASELA ELENA GÎRD[1]

[1]*University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, 6 Traian Vuia street, Bucharest, Romania*
[2] *Department of Traumatology, Technical University Munich, Klinikum rechts der Isar, Ismaninger str.22, 81675, Munich, Germany*

**corresponding author: lidiapopescu24@yahoo.com*

Manuscript received: December 2010

Abstract

Russula virescens (Greencracked Brittlegill, *Russulaceae*) mushroom is well known in Europe for its gastronomic value. The aim of our study was the determination of microscopic characteristics and chemical composition of *R. virescens* harvested from Romania. The microscopic exam revealed the presence of hyphaes with basidiospores (specific nipple - shaped spores) and hymenium with basidies in different stages of evolution. The qualitative chemical analysis showed the presence of sterols / triterpenes, non-alkaloid nitrogen compounds, aminoacids, polysaccharides (mucilages), monosaccharides and other reducing compounds. The mushroom contains 26.47 - 26.69 g% water soluble substances, 29.64 - 29.93 g% alcohool soluble substances and 13.92 - 14.48 g% polysaccharides (on a dry weight basis).

Rezumat

Specia *Russula virescens* (guşa porumbelului, vineţică), familia *Russulaceae*, este o ciupercă foarte apreciată în Europa pentru valoarea sa gastronomică. Obiectivele acestui studiu constau în stabilirea caracterelor microscopice şi a compoziţiei chimice a ciupercii *R. virescens* recoltată din România (judeţul Argeş). Microscopic au fost identificate hife cu bazidiospori (specifici fiind sporii mamelonaţi) şi himeniu cu bazidii în diferite stadii de evoluţie. Studiul chimic calitativ a relevat prezenţa de steroli/triterpene, compuşi azotaţi nealcaloidici, aminoacizi, poliholozide (mucilagii), oze şi alţi compuşi reducători. Această ciupercă conţine 26,47 - 26,69 g% substanţe solubile în apă, 29,64 - 29,93 g% substanţe solubile în alcool şi 13,92 - 14,48 g% poliholozide (rezultatele sunt raportate la substanţa uscată).

Keywords: *Russula virescens,* pharmacognostic study

Introduction

The Greencracked Brittlegill, or *Russula virescens* (Schff. ex Zart.) Fr., is an edible mushroom considered to be one of the tastiest of *Russula genus* [4, 5]. According to traditional Chinese medicine the mushroom has beneficial effects on blood lipid level regulation. Previous scientific reports, have demonstrated the cytotoxic properties of polysaccharides (from mycelial culture of *R. virescens*) upon both Sarcoma 180 and tumours [3]. Others researches indicated that the water-insoluble (1-3)-beta-D-glucan, isolated from the fresh fruiting bodies did not show antitumor activity, whilst the sulphated derivatives exhibited enhanced antitumor properties [6]. The water-soluble polysaccharides have also antioxidant properties [7]. *Russula virescens*is is wide-spread in Romania and this challenged us to initiate a pharmacognostical study on the basidiocarp, in order to examine the possible use of this mushroom in therapeutics.

Materials and Methods

The raw material consisted of aerial parts of *Russula virescens* harvested in July 2008, from Arges County (Romania). The product was naturally dried and conserved in laboratory conditions.

Specific pharmacognostical methods have been used for identity, purity and quality control [1, 2].

The identity was assessed through macroscopic exam (morphological and organoleptic characters), microscopic exam (using surface preparations clarified with a 50 g/L sodium hydroxide solution and a Zeiss Imager D1 microscope) and qualitative chemical analysis (thin layer chromatography - TLC and high performance liquid chromatography - HPLC) for triterpenes.

A spectrophotometric IR method was used in order to determine the nitrites and nitrates limits. The content of polysaccharides was investigated, using a gravimetrical method. Preliminary, the parameters "loss on drying", "water soluble-substances", "alcohol soluble-substances" and "swelling index" were also determined [9].

Parameters of TLC for triterpenes - test solution: 1 g powdered mushroom was extracted with 10 mL methanol R for 10-15 min., filtered and concentrated; TLC plates silica gel GF_{254} Merck; solvent system - hexane : ethyl acetate = 6:1 (v/v);

reference substances (Sigma; 0.1% in methanol): oleanolic acid, betulin; detection: spraying with phosphomolybdic acid 10 g / 100 mL. The chromatogram was observed in UV light (λ = 366 nm).

Parameters of HPLC for triterpenes - a HPLC Agilent 1200 equipment was used, consisting of a quaternary pump G1311A with degasificator, a detector (DAD)G1311A and a BDS Hypersil column (150 x 4.6 mm ID, 3 μm particles), with a guard-column C18 (column temperature was 25°C; wave lentgh detector λ = 215 ± 4 nm; flow: 1 mL/min; injection volume: 5 μL). The mobile phase consisted of phosphoric acid solution pH 3 (solvent A) and acetonitrile (solvent B); the ratio solvent A : solvent B was 15 : 85. The sample was prepared by refluxing the mushroom with methanol for 60 min. Oleanolic acid, ursolic acid and betulin (Sigma-Aldrich, Germany) were used as references. The methanolic extract analysis was performed at two time points: 1) immediately and 2) after 48 hours after preparation.

Parameters of IR study: a Tensor 27 FT-IR Bruker spectrometer; the KBr disk method (3 mg potassium bromide and 1.5 mg dried fruiting bodies of *R. virescens*) [8].

Statistical analysis. Statistical significance of differences between the individual treatments was evaluated by one-way ANOVA with Kruskal-Wallis post-test or Dunnett's post-test (Prism 5.01, GraphPad Software, San Diego, USA). Data are means ± SEM of at least three independent experiments.

Results and Discussion

The macroscopic examination (Figure 1) confirmed the identity of the raw material.

Figure 1.
Russula virescens (original photos)

The microscopic exam showed the presence of: mycelium with multicellular and articulated hyphaes, basidies with nipple - shaped basidiospores and hymenium with basidies in different stages of evolution (Figure 2).

The following compounds were identified in the extractive solutions, by specific chemical reactions: sterols / triterpenes (in apolar solution), sterols / triterpenes and non-alkaloid nitrogen compounds (in alcoholic solution), polysaccharides (mucilages), aminoacids, monosaccharides and other reducing compounds (in aqueosus solution). The scientific literature also mentions these active principles in *Russula* and other mushrooms [4, 6].

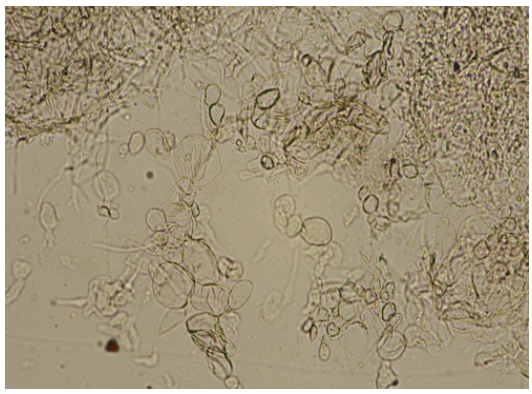
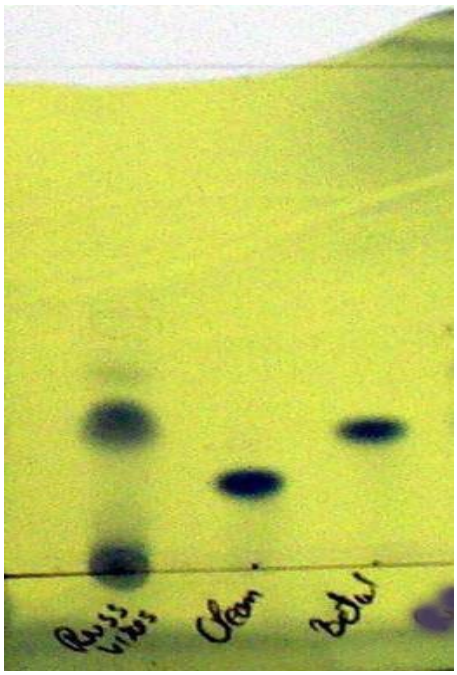


Figure 2.
Hyphaes and basidiospores of *Russula virescens*

The TLC chromatogram (Figure 3) showed the presence of three spots, with a dark blue color (after spraying with the phosphomolybdic acid reagent) corresponding to compounds with triterpenic behaviour. Nevertheless, none of the R_f values corresponded to that of the standards (oleanolic acid, betulin).



A B C

Figure 3.
TLC chromatogram of triterpenes from *Russula virescens* after spraying with phosphomolybdic acid. A) methanol extract; B) oleanolic acid; C) botulin.

The HPLC analysis (Figure 4) of the methanolic extract (immediately after preparation) showed the presence of 13 chromatographic peaks. However their retention times (T_R) were different from that of reference substances (oleanolic acid T_R = 7.607 min., ursolic acid T_R = 8.005 min. and betulin T_R = 8.252 min.).
The HPLC chromatogram of the methanolic extract (analysed after 48 hours from preparation) contains only 8 peaks (Figure 5). Compounds with the following T_R values: 1.315 min., 1.431 min., 4.138 min., 5.193 min, 8.344 min and 9.165 min. are not found any more in the extract after 48 hours. Additionally one can note the presence of a new compound with T_R = 1.264 min.
Based on these results, we concluded that some compounds of the extract are not stable in the extraction solvent (methanol).

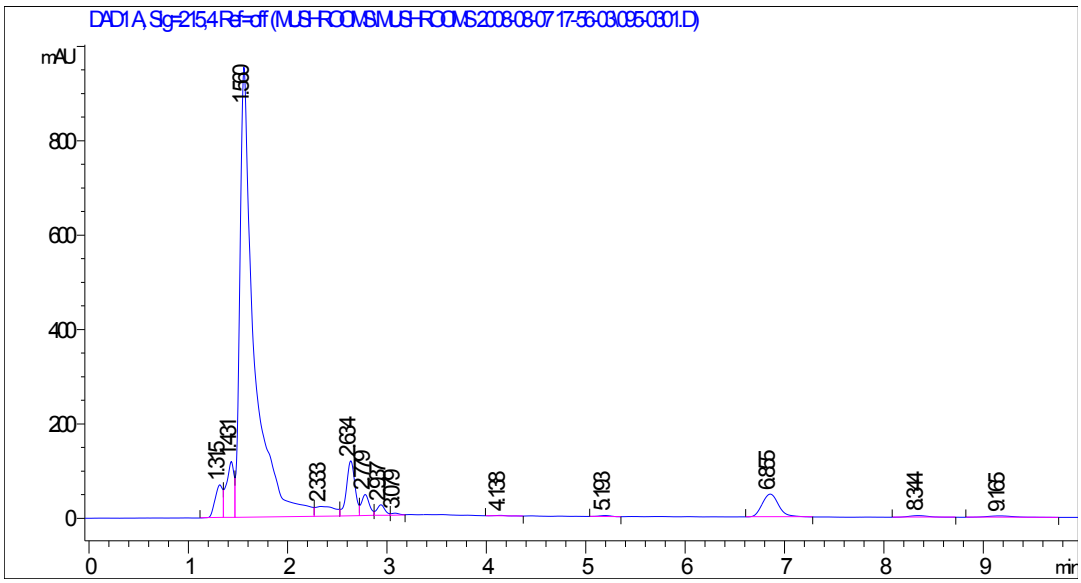


Figure 4.
HPLC chromatogram of *Russula virescens* methanolic extract, immediately after preparation

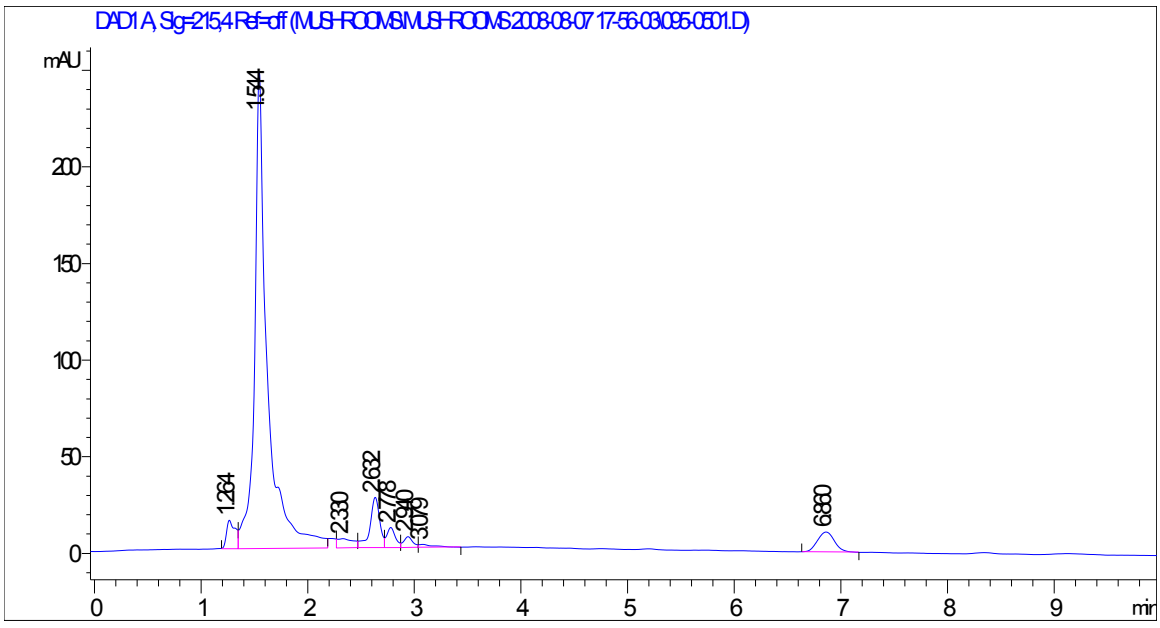


Figure 5.
HPLC chromatogram of *Russula virescens* methanolic extract, 48 hours after preparation

The infrared spectra analysis bands (range of 4000 - 500 cm^{-1}) of *R. virescens* dried fruiting bodies (Figure 6) didn't show the presence of nitrites and nitrates. Due to our results, we assume that the mushrooms were collected from a non-polluted area.

The results of the quantitative chemical analysis (calculated with reference to the dried drug) are presented in Table I. The highest content of soluble substances was obtained in alcohol. The polysaccharides content was rather high.

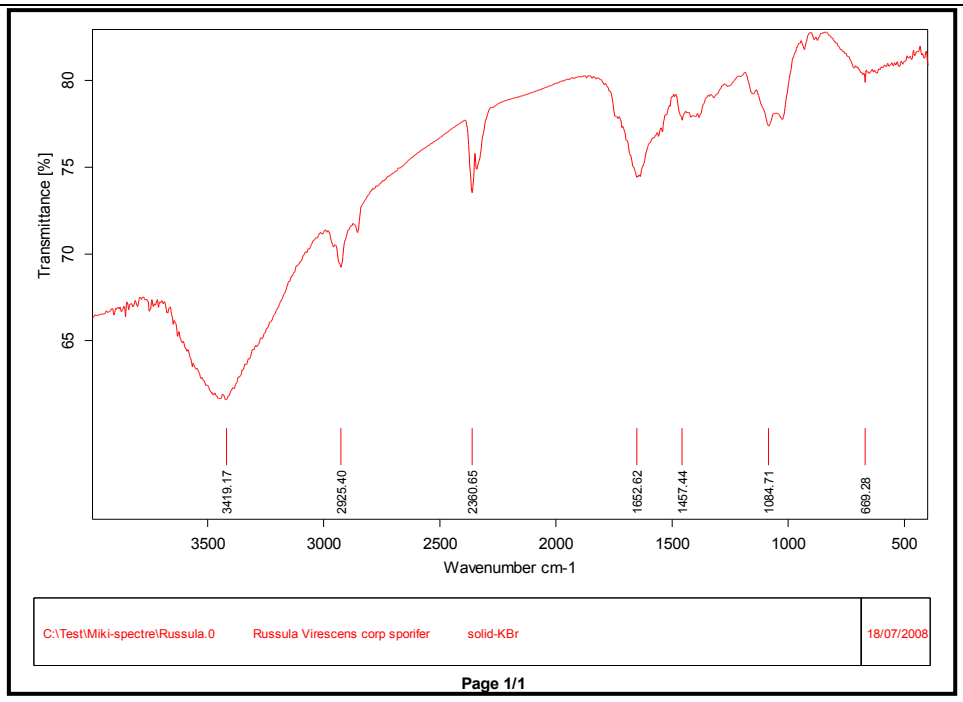


Figure 6.
The FT-IR spectra of *Russula virescens*

Table I
Results of quantitative chemical study

Determination		Results
loss on drying (g%)		8.11 – 8.37
swelling index		14.92 - 15.28
soluble substances (g%)	water	26.47 - 26.69
	alcohol	29.64 - 29.93
mucilages (g%)		13.92 - 14.48

Conclusions

The pharmacognostical screening of *Russula virescens* mushroom has been performed. The specific anatomical elements are the nipple-shaped spores. The main classes of active principles are sterols / triterpenes, non-alkaloid nitrogen compounds, polysaccharides (mucilages), monosaccharides and other reducing compounds. The HPLC analysis proved that some compounds are not stable in methanolic solutions. *Russula virescens* collected from Arges County (Romania) did not contain nitrite or nitrate salts.

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