

Determination on Antioxidant Capacity and TLC Analysis of Ten Thai *Russula* Mushroom Extracts

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Abstract

Introduction: *Russula* mushrooms have been popularly consumed as indigenous foods and used in the treatments of various diseases in the Northeastern region of Thailand for long time.

Method: This study was carried out to investigate the antioxidant capacities of selected *Russula* mushrooms and their chemical profiles. All extracts from ten *Russula* mushrooms including *R. crustosa*, *R. delica*, *R. monspeliensis*, *R. velenovskyi*, *R. virescens*, *R. lepida*, *R. alboareolata*, *R. paludosa*, *R. medullata* and *R. helios* were prepared by maceration with 95% ethanol. Their antioxidant capacities were determined on superoxide radical scavenging using photochemiluminescence (PCL) assay for both lipid-soluble and water-soluble antioxidant capacities (ACL and ACW, respectively).

Results: All *Russula* mushroom extracts exhibited antioxidant capacities in various ranges. *R. medullata* extract possessed the highest antioxidant effects in both ACL and ACW models with the antioxidant capacities of 1.1658 nmol of trolox equivalence and 1.323 nmol of ascorbic acid equivalence, respectively. Phytochemical analysis of all *Russula* mushroom extracts was conducted by thin layer chromatographic (TLC) technique. **Conclusion:** This information supports the uses of *Russula* mushrooms for health and medicinal purposes.

Keywords: *Russula*, extracts, antioxidant capacity, PCL, TLC

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1. Introduction

Russula mushrooms are the mushrooms in the family of *Russulaceae* (Jain and Pande, *et al.*, 2013). The mushroom shapes resemble of umbrella. They have clear thin cap with the gills underneath and the stem. The mushrooms are fresh, soft, fragile and perishable (Buyck, *et al.*, 2008). There are about 750 species of *Russula* distributed around the world (Joshi, *et al.*, 2012) including the United States of America, Sweden, France, Norway, Madagascar, Italy, Belgium, Taiwan, China, Japan and Thailand (Buyck, *et al.*, 2008). In Thailand, the presence of *Russula* mushrooms have been reported in 17 provinces of the Northeastern region (Jaruntorn and Chanida, *et al.*, 2010; Manassila, *et al.*, 2005). Numerous *Russula* mushrooms have been consumed as foods such as *R. monspeliensis*, *R. virescens*, *R. alboareolata*, *R. medullata*, and *R. helios* (Quiñónez-Martínez, *et al.*, 2014; Manassila, *et al.*, 2005). Some *Russula* mushrooms have the established histories of the uses in traditional medicines for the treatments of various diseases such as *R. cyanoantha* and *R. nobilis* were used for the treatments of fever, *R. luteotacta* was used for wound healing, *R. delica* and *R. parazurea* were used for the treatments of gastritis and high blood pressure while *R. acrifolia* was used for treatments of skin cancer (Sutachit, *et al.*, 2002). Moreover, some *Russula* mushrooms have also been traditionally used as tonic such as *R. cyanoxantha*, *R. nobilis*, *R. delica*, *R. parazurea*, *R. acrifolia* and *R. luteotacta* (Sanmeea, *et al.*, 2002). In addition, *Russula luteotacta* has been used as sleep promoting agent (Sanmeea, *et al.*, 2002). However, there

are some reports about side effects and toxicities of *Russula* mushrooms. *Russula densifolia*, *R. fragtissima* and *R. rosacea* can cause gastroenteritis while *R. olivacea* can cause nausea, vomiting and diarrhea (David, *et al.*, 1942). Moreover, *R. subnigricans* can cause rhabdomyolysis, severe electrolyte disturbance (hypocalcemia), respiratory failure, acute renal failure, pulmonary edema, ventricular tachycardia, and circulatory shock (Po-Tsang Lee, *et al.*, 2001).

Biological activities of some *Russula* mushrooms have been previously reported. *R. delica* showed antimicrobial activities against various bacteria and fungi including *Salmonella enteritidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Micrococcus flavus*, *Bacillus cereus* and *Candida albicans* (Turkoglu, *et al.*, 2007). *Russula griseocarnosa*, *R. albonigra*, *R. laurocerasi* and *R. delica* exhibited antioxidant activities tested by various *in vitro* assays such as reducing power, hydroxyl radical scavenging, chelating ability of ferrous ion, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, superoxide radical scavenging and b-carotene bleaching assays (Dasgupta, *et al.*, 2014; Khatua, *et al.*, 2013; Chen, *et al.*, 2010). Water extract of *R. paludosa* showed inhibitory effect to HIV-1 reverse transcriptase (Wang, *et al.*, 2007) while *R. lepida* exhibited antiproliferative activity to hepatoma Hep G2 cells and human breast cancer MCF-7 cells (Zhang, *et al.*, 2010). *Russula virescens* exhibited the anti-inflammatory effect in the RAW 264.7 cell by suppressed expression of STATs, reduction of TNF- α and NO production (Hur, *et al.*, 2012).

Some chemical constituents have been reported from *Russula* mushrooms. Several phenolic acids such as *p*-hydroxy-benzoic acid, chlorogenic acid, ferulic acid, and coumaric acid and some flavonoids such as chrysin and catechin were reported from *R. delica* along with oleanolic acid (Kalogeropoulos, *et al.*, 2013; Yaltirak, *et al.*, 2009). *Russula griseocarhosa* composed of caffeic acid, protocatechuic acid and quercetin (Chen, *et al.*, 2010). Various terpenoids were found in *R. lipida*, *R. foetens* and *R. amarissima* including aristolane and marasmane aristolane sesquiterpenoids (Jian-Wen, *et al.*, 2000, Clericuzio, *et al.*, 2012).

Even though many *Russula* mushrooms have been used as foods and for the treatments of various diseases in the Northeastern part of Thailand for a long time, however, there are some *Russula* mushrooms in this area that still have never been studied on their biological properties and phytochemistry. Therefore, this experiment was set up in order to investigate the *in vitro* antioxidant capacity of the extracts from ten selected *Russula* mushrooms using photochemiluminescence (PCL) assay. Phytochemical analysis of all *Russula* mushroom extracts was conducted by thin layer chromatographic (TLC) techniques.

2. Materials and Methods

Chemicals and equipment

Kit for lipid-soluble (ACL) and water-soluble antioxidant capacities (ACW) were purchased from Analytik Jena, Germany (Jena, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma (St. Louis, MO, USA). Acetic acid (glacial) 100%, sulfuric acid 95-97% and TLC silica gel 60 GF₂₅₄ 20x20 cm were purchased from Merck (Darmstadt, Germany). Ethanol 95%, ethylacetate and methanol were purchased from Lab scan Ltd (Bangkok, Thailand). Caffeic acid and 3,4-dicaffeoylquinic acid were purchased from Chromadex (Irvine, CA, USA).

Samples of *Russula* Mushrooms

Ten different *Russula* mushrooms (Figure 1.) including *R. crustosa*, *R. delica*, *R. monspeliensis*, *R. velenovskyi*, *R. virescens*, *R. lepida*, *R. alboareolata*, *R. paludosa*, *R. medullata* and *R. helios* were collected from Kalasin, Mukdahan, Sakon Nakhon, and Yasothon provinces in the Northeastern part of Thailand in May - October, 2013 to June - September, 2014. All mushrooms were botanically identified by Mr. Winai Klinhom, the mushroom specialist of Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand.

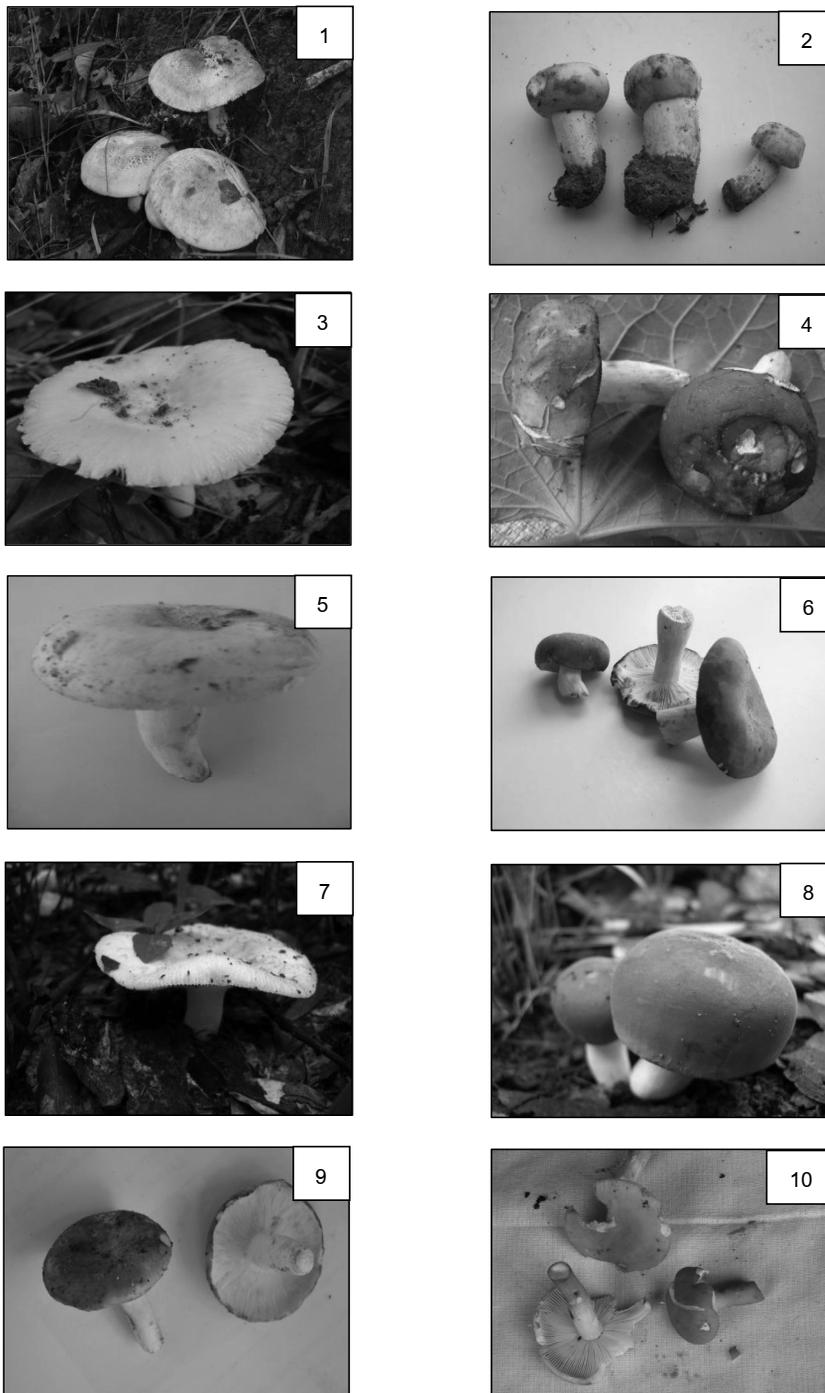


Figure 1 : *Russula* mushrooms used in the study: *R. crustosa* (1), *R. delica* (2), *R. monspeliensis* (3), *R. velenovskyi* (4), *R. virescens* (5), *R. lepida* (6), *R. alboareolata* (7), *R. paludosa* (8), *R. medullata* (9) and *R. helios* (10).

Preparation of mushroom extracts

Ten collected fruiting body of *Russula* mushrooms were separately dried in hot air oven at 50°C for about 18-20 hours until dried then they were ground into powder using an electronic grinder. The powder samples of each *Russula* were separately subjected to extraction by maceration method. Briefly, 100 grams of dried *Russula* powder was mixed with 1000 ml 95% ethanol and sonicated for 1 hour. The ethanolic extract solution was filtered and evaporated using the rotary evaporator to yield dried *Russula* extracts. All extracts were stored at -20°C until use.

Determination of antioxidant capacity

The antioxidant capacity of all ten *Russula* mushroom extracts was determined on superoxide radical scavenging activity using photochemiluminescence (PCL) assay kit by the PhotochemÒ (Analytik Jena, Germany). Briefly, superoxide anion radicals ($O_2^{\cdot -}$) were generated in the system by optical excitation or irradiation of luminol which was a photosensitizer substance. The antioxidant capacities of samples were measured by their inhibitory effects on luminescence generation, compared with the standard antioxidant (constructed calibration curves). Antioxidative capacity of lipid soluble substances (ACL) and antioxidative capacity of water soluble substances (ACW) assays were performed using their reagent kits and following the instruction described by the supplier (Analytik Jena, Germany). The PCL measurement was done 100 µg each *Russula* mushroom extracts. The

antioxidant capacity of lipid soluble substances were measured with ACL kit. For each measurement, the antioxidant reaction was initiated by adding various concentrations (10, 20 and 30 µl) of standard antioxidant compound (trolox) or test samples (*Russula* mushroom extracts) to the mixture of 2,300 µl of dilution reagent 1 (methanol), 200 µl of reaction buffer (reagent 2) and 25 µl of protosensitizer (reagent 3). The antioxidant capacity of water soluble substances were measured with ACW kit. The antioxidant reaction was initiated by adding various concentrations (10, 20 and 30 µl) of standard antioxidant compound (ascorbic acid) or test samples (*Russula* mushroom extracts) 1,500 µl reagent 1 (buffer solution), 1,000 µl reagent 2 and 25 µl reagent 3 (photosensitizer). The results were presented in equivalent unit (nmol) of ascorbic acid for ACW system and trolox (synthetic vitamin E derivative) equivalent unit for ACL system. All determinations were conducted in duplicate then the average and standard deviation of the results were calculated.

Phytochemical analysis

All extracts from *Russula* mushrooms were analyzed by thin layer chromatography on TLC pre-coated silica gel 60 GF₂₅₄ plate using ethylacetate: acetic acid: formic acid: water (137: 11: 11: 26) as a solvent system. TLC plates were detected under UV254 and 366 nm and a DPPH spray reagent.

Statistical analysis

Statistical analysis was performed using SPSS for window version 21.0 software program

(SPSS Inc., USA.). The average values were calculated and compared using independent-sample t test analysis. The significant difference was set at the level of $p < 0.05$.

3. Results and Discussion

The yields of ethanolic extracts from ten selected *Russula* mushrooms were in the range of 10 – 25 % w/w. Some mushroom extracts appeared as sticky dark brown semi-solid and specific odor however, *R. velenovskyi* appeared as sticky light brown semi-solid, specific odor as shown in Table 1.

Table 1 Percentage (yield) and physical characteristics of ten selected *Russula* mushroom extracts

<i>Russula</i> sample	Yield (% w/w)	Physical characteristic of the extract
<i>R. crustosa</i>	16.35	sticky dark brown semi-solid, specific odor
<i>R. delica</i>	17.76	sticky dark brown semi-solid, specific odor
<i>R. monspeliensis</i>	22.76	sticky dark brown semi-solid, specific odor
<i>R. velenovskyi</i>	14.58	sticky light brown semi-solid, specific odor
<i>R. virescens</i>	16.86	sticky dark brown semi-solid, specific odor
<i>R. lepida</i>	15.99	sticky dark brown semi-solid, specific odor
<i>R. alboareolata</i>	18.96	sticky dark brown semi-solid, specific odor
<i>R. paludosa</i>	15.16	sticky dark brown semi-solid, specific odor
<i>R. medullata</i>	10.54	sticky dark brown semi-solid, specific odor
<i>R. helios</i>	13.13	sticky dark brown semi-solid, specific odor

Antioxidant capacity of *Russula* extracts

Ten *Russula* mushroom ethanolic extracts at the concentration of 100 mg were determined on superoxide radical scavenging activity using photochemiluminescence assay. As shown in Table 2, the overall antioxidant capacity in ACL system ranged from 0.191 to 1.166 nmol of trolox. Even though there was no significant difference between the antioxidant capacities in ACL system of all extracts, however, the extract from *R. medullata* possessed the highest antioxi-

dant activity of 1.166 nmol trolox equivalence, followed by extracts of *R. alboareolata* and *R. helios* (0.844 and 0.788 nmol trolox equivalence, respectively) while the extract from *R. paludosa* promoted the lowest antioxidant effect with antioxidant capacity of 0.191 nmol trolox equivalence. In ACW system, the antioxidant capacity of ten *Russula* extracts ranged from 0.269 to 1.323 nmol of ascorbic acid equivalence. Similar to ACL system, there was no significant difference between the antioxidant capacities in ACW

system of all extracts. The extract with the highest antioxidant activity was also *R. medullata* extract which promoted the capacity of 1.323 nmol of

ascorbic acid equivalence, followed by extracts of *R. alboareolata* and *R. lepida* (1.247 and 0.940 nmol ascorbic acid equivalence, respectively).

Table 2 Antioxidant capacity in lipid phase (ACL) and water phase (ACW) of ten *Russula* mushroom extracts by PCL assay

<i>Russula</i> sample	Antioxidant capacity (nmol)*	
	ACL (trolox equivalence)	ACW (ascorbic acid equivalence)
<i>R. crustosa</i>	0.420 ± 0.197	0.832 ± 0.327
<i>R. delica</i>	0.488 ± 0.391	0.840 ± 0.320
<i>R. monspeliensis</i>	0.275 ± 0.133	0.269 ± 0.131
<i>R. velenovskyi</i>	0.459 ± 0.090	0.386 ± 0.181
<i>R. virescens</i>	0.393 ± 0.117	0.709 ± 0.274
<i>R. lepida</i>	0.562 ± 0.239	0.940 ± 0.433
<i>R. alboareolata</i>	0.844 ± 0.050	1.247 ± 0.506
<i>R. paludosa</i>	0.191 ± 0.130	0.428 ± 0.154
<i>R. medullata</i>	1.166 ± 0.008	1.323 ± 0.344
<i>R. helios</i>	0.788 ± 0.049	0.884 ± 0.204

*Results were expressed as Mean ± SD (n=2). There is no significant difference between samples.

Phytochemical analysis of *Russula* mushroom extracts by TLC

As shown in Figure 2, all *Russula* mushroom extracts showed similar specific chromatographic fingerprints with dark quenching and fluorescence chromatographic bands under the detection of UV 254 and 366 nm, respectively suggesting the presences of chromophores. After detection with DPPH spray reagent, most extracts including the extracts from *R. medullata* and *R. alboareolata* promoted the pale yellow bands on the purple background suggesting the presence of antioxidative compounds.

Antioxidants can be classified into two main divisions depending on their water preferable solubility (hydrophilic) or lipid preferable solubility (lipophilic). In general, water soluble antioxidants can react with free radicals or oxidants in the cell cytosol and the blood plasma, while lipid soluble antioxidants protect cell membranes from lipid peroxidation (Sies, et al., 1997). This study demonstrated that among ten selected *Russula* extracts, the extracts from *R. medullata* and *R. alboareolata* possessed the highest antioxidant capacity against superoxide anion ($O_2^{\cdot-}$) radicals in both ACL and ACW systems. These

extracts along with extracts from other *Russula* mushroom samples showed specific TLC fingerprints with the presences of antioxidant compounds. The results indicated the antioxidant potentials of Thai *Russula* mushrooms. These two *Russula* extracts could be further developed as

new alternative sources of natural antioxidants for the protection or treatment of free radical-related diseases as well as for the development as nutraceutical products in the future. Further studies on other related pharmacological activities and toxicity evaluations should be conducted.

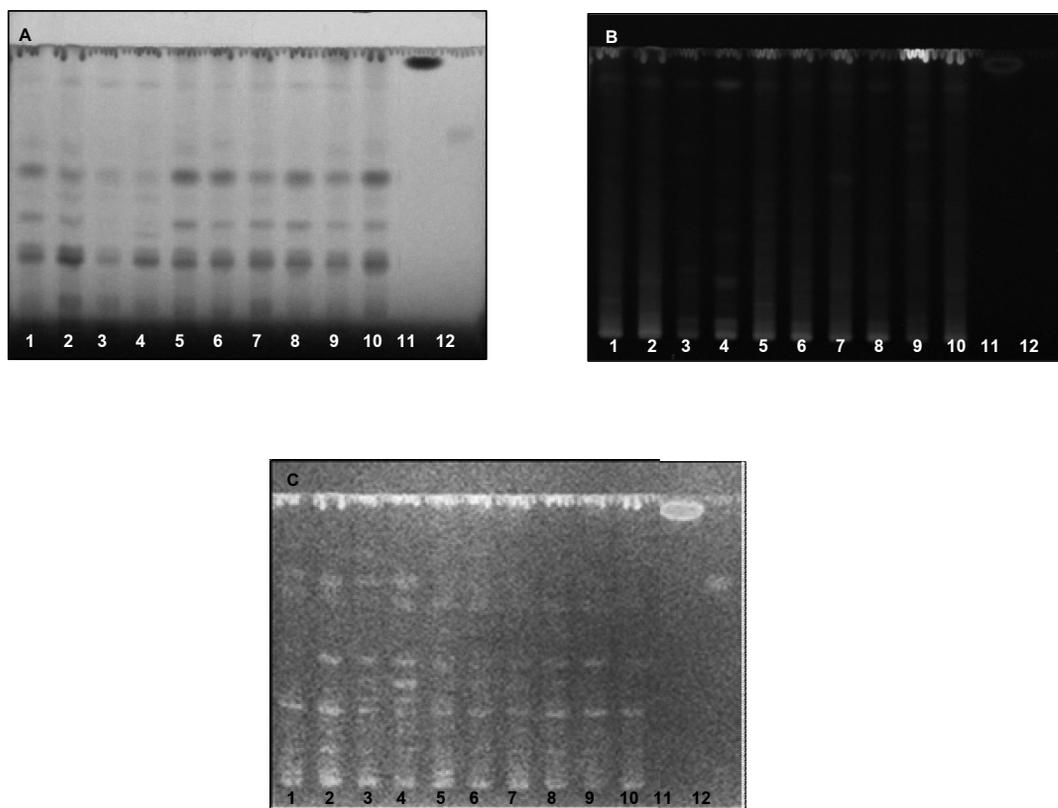


Figure 2 : TLC fingerprints of ethanolic extracts from *Russula* mushrooms; stationary phase: silica gel 60 GF₂₅₄, solvent system: ethylacetate: acetic acid: formic acid: water (137: 11: 11: 26), track: 1= *R. crustosa*, 2 = *R. delica*, 3 = *R. monspeliensis*, 4 = *R. velenovskyi*, 5 = *R. virescens*, 6 = *R. lepida*, 7 = *R. alboareolata*, 8 = *R. paludosa*, 9 = *R. medullata*, 10 = *R. helios*, 11 = caffeic acid, 12 = 3,4-dicaffeoylquinic acid; (A) = UV 254nm, (B) = UV 366nm, (C) = DPPH spray reagent.

4. Conclusion

Regarding this current study, we firstly described the antioxidant capacities of ten *Russula* mushrooms of Thailand with their TLC fingerprints. The studies on phytochemical components, apoptotic and cytotoxic properties of these *Russula* mushrooms have been currently carried out.

5. Acknowledgements

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