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Quantification of Active Compounds of Edible Mushrooms in University of Phayao, Thailand

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Abstract: The purpose of this research was to quantify active compounds from sixteen species of edible mushrooms in University of Phayao, Thailand. Antioxidant and free radical scavenging activity of the polysaccharides extracted from edible mushrooms were evaluated with 3 methods including Thin-layer chromatography (TLC) fingerprint method, DPPH radical scavenging capacity assay and high-performance liquid chromatography (HPLC). Analysis of these extracts by TLC fingerprint and DPPH from sixteen species of edible mushrooms were found alkaloid, tannin, steroid and antioxidant, but 2 species in *Lacterius piperatus* (Scop. Ex Fr.) S.F. Gray and *Russula cyanoxantha* (Schaeff.) Fr. were not found alkaloids and tannin. According to the type and quantity of phenolic compounds in the edible mushrooms by HPLC technique, the specimens consisted of oxalic acid, tartaric acid, malic acid, quinic acid, and succinic acid.

Key words: mushrooms, active compounds, University of Phayao

1. Introduction

Mushroom consumption nowadays is popular in Thailand with the mushrooms are cheap, and safe consumption because the pesticides are minimal or nothing [1]. Mushrooms are high in proteins, vitamins, and minerals but those are low fat [2]. There are high nutritional values that contain many nutrients such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), folate (B9) [3], dietary fibers including vitamin C, D [4]. In addition, mushrooms are important because of their medicinal properties such as preventing and strengthening the immune system [5], hypoglycemic and hypolipidemic activity [4] as well as mushrooms are the source of several secondary metabolites that have the property of antioxidants such as

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phenolic compounds, ergothioneine and saccharide [6]. Moreover, several species of mushrooms are active against the human pathogen, cancer, diabetes, hypertension, hypercholesterolemia condition including tumor [2]. However, some mushrooms are caused by food poisoning. Poisonous mushrooms are identified based on morphological characteristics that they must identify and know it before cooking or applying [7].

The thin layer chromatography (TLC) is the one in various methods that are available for the screening of pharmacologically active substances in extracts. This is a simple, quick reliable and inexpensive procedure that can be used for screening of plant extracts [8], effectively quality evaluation of the plant or its derived herbal products. Thin-layer chromatography enables analysts to separate and determine useful natural products in complex mixtures of plant products. Various chromatographic systems useful for the identification; separation and quantification [9]. The current study presents the application of chemometrics to high performance thin-layer chromatography (HPTLC) fingerprints of medicinal mushrooms [10].

Nevertheless, most of the studies always are the study of economic mushrooms. Therefore, the purpose of this research is the study of properties in edible mushrooms which are wild mushrooms in University of Phayao, Thailand. Those are mushrooms that are popular in consumption and available in Thailand by analyzing phenolic content, antioxidant activity test, DPPH, and study on HPLC.

2. Materials and Methods

2.1 Mushroom specimens

The samples were divided out 16 mushroom specimens and 1 unknown by 16 mushroom species were collected from University of Phayao that were as follows: *Amanita princeps* Corner & Bas. (specimen code UP_001), *A. hemibapha* (Berk. et Br.) Sacc. subsp. javanica Corner et Bas. (specimen code UP_002), *Russula alboareolata* Hongo. (specimen code UP_003), *R. delica* Fr. (specimen code UP_004), *R. luteotacta* Rea. (specimen code UP_005), *R. aeruginea* Lindbl. (specimen code UP_006), *R. virescens* Fr. (specimen code UP_007), *Lactarius volemus* Fr. (specimen code UP_008), *Boletus reticulatus* Schaeff. (specimen code UP_009), *Trichaptum abietinum* (Dicks.:Fr.) Ryv. (specimen code UP_0010), *Cantharellus minor* Peck. (specimen code UP_0011), *Craterellus oderatus* (Schw.) Fr. (specimen code UP_0012), *Lentinus polychrous* Lev. (specimen code UP_0013), *Ganoderma ludidum* (Fr.) Karst. (specimen code UP_0014), *Filoboletus manipularis* (Berk.) Sing. (specimen code UP_0015), and *Phylloporus bell* (Mass.) Corner (specimen code UP_0016).

2.2 Preparation method for a mushroom extract solution

By weighed in 30 mg of mushroom specimens and dissolved in 30 mL of 95% alcohol.

2.3 Identify the method of Thin Layer Chromatography (TLC chromatogram)

First, 20 µL of each mushroom extract was spotted onto 10 µL of TLC silica gel 60 GF254 Glass plates 20x10 cm using CAMAG Automatic TLC Sampler 4, band sizes of 0.6 cm by spaced from below and both side of 1.5 cm. Second, the TLC plate was brought to development procedure in chromatographic tank using the mobile phase as dichloromethane-ethyl acetate-petroleum ether-formic acid-ethanol (8:3:9:0.8:0.5) by mobile phase of substance was 8 cm. Third, the TLC plate was brought to observe the chromatogram characteristics of the extracted substance by examining under the ultraviolet radiation (UV) at 254 and 366 nm irradiation wavelengths and spraying with the inspection solution. Fourth, the chromatogram data were recorded by digital cameras.

2.4 Preparation method of spray reagent according to Wagner and Bladt (1996)

• *anisaldehyde-Sulphuric acid reagent*

0.5 mL anisaldehyde was dissolved in 10 mL concentrated sulfuric acid, added 85 mL methanol, added 5 mL concentrated sulfuric acid and brought to spray after that baked at 105 °C for 5-15 minutes (for quantified the substance of terpenoid group).

• *dragendorff's reagent*

Solution A (basic bismuth nitrate was dissolved in 10 mL glacial acetic acid and 40 mL water) mixed with solution B (8 g potassium iodide was dissolved in 30 mL water), by 1:1 ratio (Stock solution). Before it was used, 1 mL the stock solution had mixed with 2 mL glacial acetic acid and 40 mL water (for quantified the substance of alkaloids group) [11].

2.5 Analysis methods of organic acid by modifying the method according to Ribeiro et al. (2008)

The mushroom specimens were mashed thoroughly. Then, mashed specimens were weighed of 1 g and added 10 mL deionized water (DI water). Next, brought it to vortex for 1 minute and heated at 100 °C by hot air oven for 15 minutes. After that, set aside to cool down and filtered it with filter paper no. *1*. Finally, filtered it with filter paper 0.2 µm and brought it to analyze the acid content with high-performance liquid chromatography (HPLC) [12].

2.6 Analysis conditions of organic acid using HPLC by modifying the method according to Qiu, J. (1999) [13] and Guide to aminex HPLC columns book according to Wagner and Bladt (1996) [11]

Separation of chemical compound with HPLC by using the Biorad column version Aminex HPX-87H (300 mm×7.8 mm I.D.), with guard column (30 mm×4.6 mm I.D., Bio-Rad, CA, USA). The mobile phase was the isocratic system. Mobile phase A was 0.018 N. Sulfuric acid in DI water, water, a temperature that has flow rate at 0.7ml/minute, the column of 65 ˚C, an Injection volume of 20 µl. Measurement of the absorbance by the photodiode array detector at a wavelength of 220 nm and classification the composition of the substance to comparison with the standard substance.

3. Results and Discussion

From the examination of mushroom extracts by the TLC fingerprint method using a detection reagent. The reagent of the alkaloids group was dragendorff 's reagent, antioxidant activity group was DPPH, steroid group was 10% sulphuric acid, tannin group was 0.2% ferric chloride and terpene group was anisaldehyde-sulphuric acid reagent. the examination of active compound in mushroom extracts by TLC fingerprint method of mushroom specimens extract with 20µL, 95% ethyl alcohol using mobile phase as dichloromethane-ethyl acetate-petroleum ether-formic acid-ethanol (8:3:9:0.8:0.5), after that examined under the ultraviolet radiation (UV) at 254 nm, 366nm irradiation wavelength and daylighting as shown in figures 1-3.

The analysis result of chemical compositions were found in mushroom amounts of 16 species that were specimen codes as UP_001, UP_002, UP_003, UP_004, UP_005, UP_006, UP_007, UP_008, UP_009, UP_0010, UP_0011, UP_0012, UP_0013, UP_0014, UP_0015, and UP_0016 found that every extracted mushroom specimen contained substance of steroid and terpene but alkaline and tannin were not found in any extracted specimens. Moreover, antioxidant activities were found in four extracted mushroom species that were UP_005 (*R. luteotacta* Rea.), UP_007 (*R. virescens* Fr.), UP_0014 (*Ganoderma ludidum* (Fr.) Karst.) and UP_0016 (*Phylloporus bell* (Mass.) Corner) as shown in Table 1.

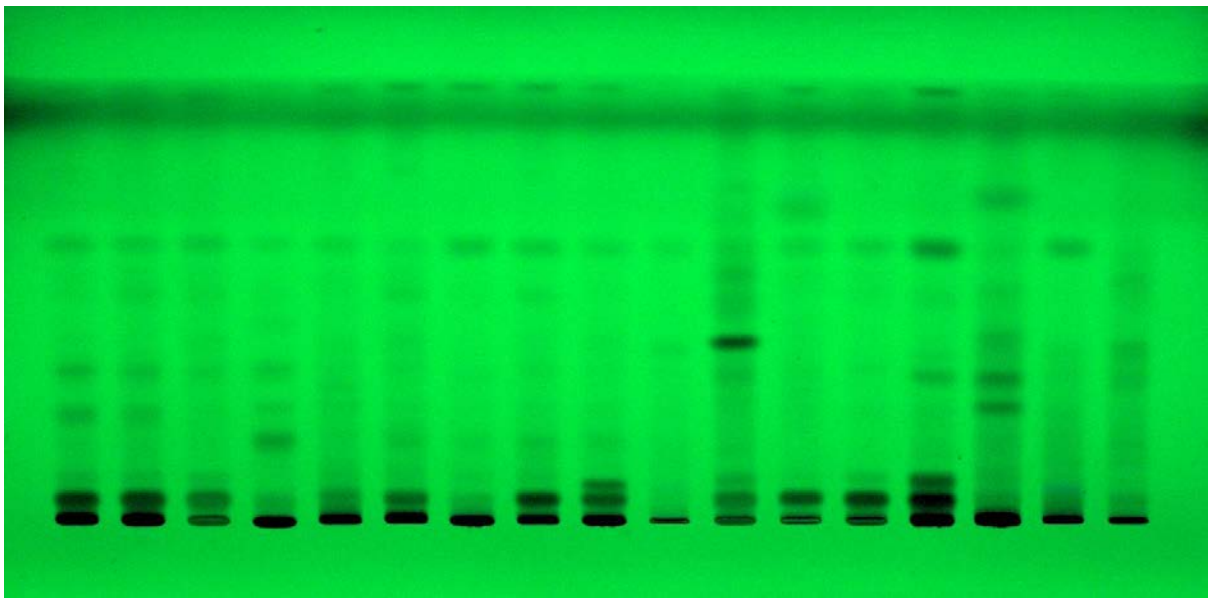

Fig. 1 Characteristics of Thin Layer Chromatography (TLC chromatogram) of mushroom specimens extract with 20µL, 95% ethyl alcohol using mobile phase as dichloromethane-ethyl acetate-petroleum ether-formic acid-ethanol (8:3:9:0.8:0.5) after that examined under the ultraviolet radiation (UV) at 254 nm irradiation wavelength.

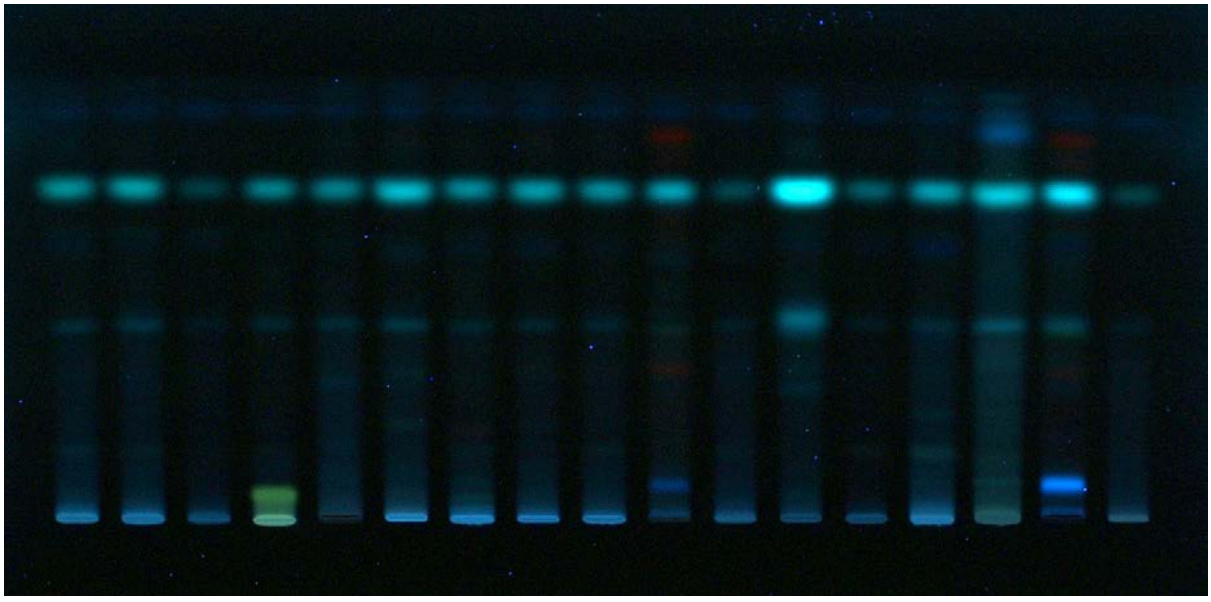

Fig. 2 Characteristics of Thin Layer Chromatography (TLC chromatogram) of mushroom specimens extract with 20µL, 95% ethyl alcohol using mobile phase as dichloromethane-ethyl acetate-petroleum ether-formic acid-ethanol (8:3:9:0.8:0.5) after that examined under the ultraviolet radiation (UV) at 366 nm irradiation wavelength.

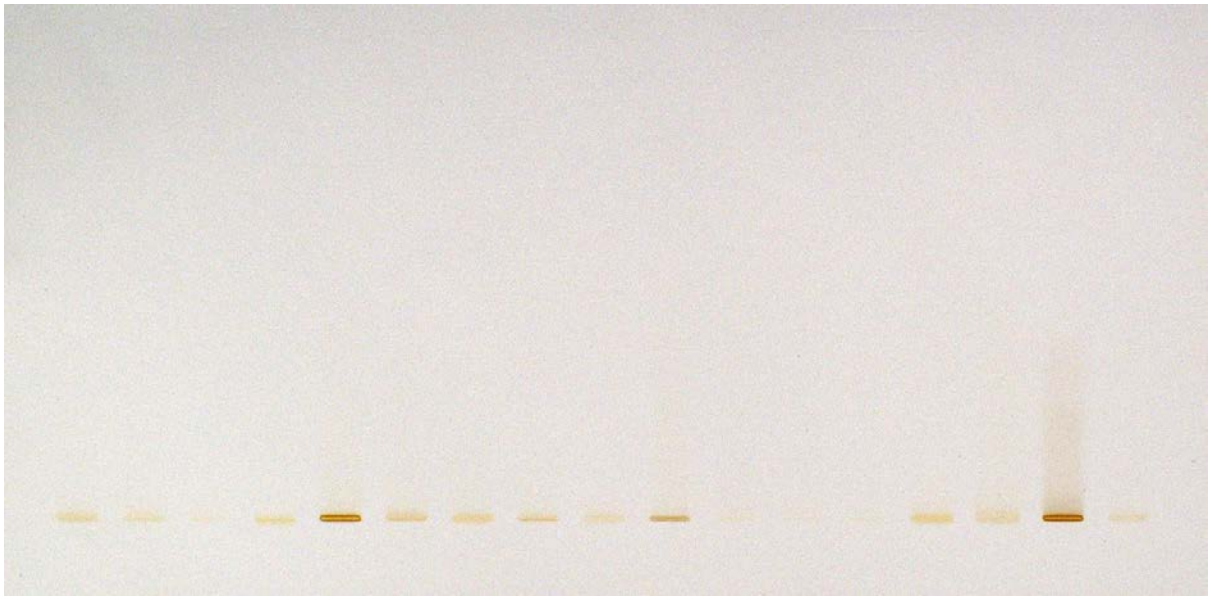

Fig. 3 Characteristics of Thin Layer Chromatography (TLC chromatogram) of mushroom specimens extract with 20µL, 95% ethyl alcohol using the mobile phase as dichloromethane-ethyl acetate-petroleum ether-formic acid-ethanol (8:3:9:0.8:0.5) after that examined under daylighting.

Table 1 Chemical compositions were found in mushroom specimens.

Mushroom specimens	alkaloids	antioxidant	steroid	tannin	terpene
UP_001	-	-	+	-	+
UP_002	-	-	+	-	+
UP_003	-	-	+	-	+
UP_004	-	-	+	-	+
UP_005	-	+	+	-	+
UP_006	-	-	+	-	+
UP_007	-	+	+	-	+
UP_008	-	-	+	-	+
UP_009	-	-	+	-	+
UP_0010	-	-	+	-	+
UP_0011	-	-	+	-	+
UP_0012	-	-	+	-	+
UP_0013	-	-	+	-	+
UP_0014	-	+	+	-	+
UP_0015	-	-	+	-	+
UP_0016	-	+	+	-	+

Remark: (-) Substances were not found, (+) Substances were found from the examination.

The analysis results of organic acid including oxalic acid, tartaric acid, malic acid, quinic acid and succinic acid (µg/g) in mushroom amounts of 16 species by HPLC found that oxalic acid contents were the highest in UP_008 (*Lactarius volemus* Fr.), UP_007 (*R. virescens* Fr.) and UP_009 (*Boletus reticulatus* Schaeff), respectively. Tartaric acid contents were the highest in UP_0010 (*Trichaptum abietinum* (Dicks.:Fr.) Ryv), UP_005 (*R. luteotacta* Rea.) and UP_009 (*Boletus reticulatus* Schaeff), respectively. Malic acid contents were the highest in UP_007 (*R. virescens* Fr.), UP_0010 (*Trichaptum abietinum* (Dicks.:Fr.) Ryv) and UP_0011 (*Cantharellus minor* Peck.), respectively. Quinic acid contents were the highest in UP_007 (*R. virescens* Fr.), UP_0014 (*Ganoderma ludidum* (Fr.) Karst.) and UP_009 (*Boletus reticulatus* Schaeff), respectively. Succinic acid contents were the highest in UP_0011 (*Cantharellus minor* Peck.), UP_005 (*R. luteotacta* Rea.) and UP_0014 (*Ganoderma ludidum* (Fr.) Karst.), respectively. As shown in Table 2.

However, the analysis results of organic acid content in table 2 that was not detected including oxalic acid, tartaric acid, malic acid, quinic acid and succinic acid in mushroom amounts from 16 species by HPLC were as follows: UP_001 (*Amanita princeps* Corner & Bas.), UP_002 (*A. hemibapha* (Berk. et Br.) Sacc. subsp. javanica Corner et Bas.), UP_004 *(R. delica* Fr.), and UP_006 (*R. aeruginea* Lindbl.) were not detected Quinic acid. UP_003 (*Russula alboareolata* Hongo.) was not detected Tartaric acid and Quinic acid. UP_0010 (*Trichaptum abietinum* (Dicks.:Fr.) Ryv.) was not detected Oxalic acid, Quinic acid, and Succinic acid. UP_0012 (*Craterellus oderatus* (Schw.) Fr.) was not detected Oxalic acid. UP_0013 *(Lentinus polychrous* Lev.) was not detected Tartaric acid, Malic acid, Quinic acid, and Succinic acid. UP_0014 (*Ganoderma ludidum* (Fr.) Karst.) was not detected Tartaric acid and Malic acid. And UP_0015 (*Filoboletus manipularis* (Berk.) Sing.) and UP_0016 (*Phylloporus bell* (Mass.) Corner) was not detected any organic acid.

Table 2: The results of GABA (µg/g) were studied on HPLC.

	organic acid content (µg/g sample)				
Specimens	Oxalic acid	Tartaric acid	Malic acid	Quinic acid	Succinic acid
UP_001	444.84±8.34	2832.55±12.35	4850.41±14.04	ND	4375.63±86.18
UP_002	1363.48±6.54	1713.95±12.45	10002.24±21.56	ND	8941.49±78.57
UP_003	373.26±12.76	ND	2181.26±69.80	ND	35822.41±16.14
UP_004	512.1±5.56	3132.07±40.85	20713.68±88.71	ND	18060.98±67.15
UP_005	1353.08±31.81	4892.61±58.49	22358.82±60.52	9555.17±44.55	53098.13±71.46
UP_006	1605.4±45.00	3194.75±63.43	22624.94±119.34	ND	17703.60±71.73
UP_007	1699.1±5.57	2131.15±39.40	31957.51±56.62	33871.67±58.50	24404.59±72.95
UP_008	2061.94±112.95	1795.42±21.27	9564.85±91.54	9611.68±30.93	5948.91±66.76
UP_009	1686.01±88.57	4572.74±58.96	9562.45±68.28	15905.47±73.85	9259.71±63.81
UP_0010	ND	20248.67±48.14	26902.03±94.33	ND	ND
UP_0011	1268.69±3.01	1416.24±57.84	23529.44±170.38	10321.57±65.81	71340.35±85.46
UP_0012	ND	1176.22±60.24	3672.40±48.75	7335.13±56.97	11466.05±23.53
UP_0013	1089.02±27.76	ND	ND	ND	ND
UP_0014	404.18±18.57	ND	ND	20874.96±37.43	42474.85±75.32
UP_0015	ND	ND	ND	ND	ND
UP_0016	ND	ND	ND	ND	ND

Remark: (ND) Not detected

From studying on antioxidant and free radical scavenging activity of the polysaccharides extracted from edible mushrooms were evaluated with 3 methods including Thin-layer chromatography (TLC) fingerprint method, DPPH radical scavenging capacity assay and high-performance liquid chromatography (HPLC). Analysis of these extracts by TLC fingerprint and DPPH from sixteen species of edible mushrooms were found alkaloid, tannin, steroid, and antioxidant. the results of this research were had in some ways consistent with the results or methodology of the other studies.

Wannet et al. (2000) have also studied HPLC detection of soluble carbohydrates involved in mannitol and trehalose metabolism in the edible mushroom (*Agaricus bisporus*) which detected various types of carbohydrates (polyols, mono- and disaccharides, and phosphorylated sugars) and was used to determine the

levels of carbohydrates involved in mannitol and trehalose metabolism in the edible mushroom (*Agaricus bisporus*) [14].

Barros et al. (2007) have also studied fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms (*Agaricus arvensis*, *Lactarius deliciosus*, *Leucopaxillus giganteus*, *Sarcodon imbricatus*, *Tricholoma portentosum*) from Northeast Portugal that evaluated chemical composition included moisture, total oil content, crude protein, ash, carbohydrates, and nutritional value determination. The wild mushrooms were rich sources of protein and carbohydrates and had low amounts of fat. The composition in individual sugars was also determined by HPLC coupled to a refraction index detector found that mannitol and trehalose were the most abundant sugars [15].

Armassa et al. (2009) have studied the antioxidative activity and cytotoxic effect in breast cancer cell line (MCF-7) of *Lentinus polychrous* Lev. and *Ganoderma lucidum* (Fr.) Karst. were correspondingly determined using the 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) which shows an extract from edible *L. polychrous* Lev. exhibited similar antioxidative activity and the total phenolic compounds to the *G. lucidum* extracts. *L. polychrous* Lev. possessed radical scavenging activity and the total phenolic content was not different from the *G. lucidum* extract [16].

Altuntas and Aslim (2011) have also studied HPLC Analysis of phenolic compounds in two edible mushrooms (*Auricularia auricula*-judae (Bull.) J.Schrot. and *Pleurotus eryngii* (DC.) Quel.) extracts and their protective effect against oxidative damage in BHK-21 cell line are two edible mushrooms they have many biological activities that were investigated the phenolic composition, protective cytotoxic effects, and analysis of phenolic compounds in these edible mushrooms species has been carried out by HPLC coupled to photodiode array detector (HPLC-DAD) [17].

Rattana and Sangsanga (2016) have studied the antioxidant and prebiotic properties studied nine wild edible mushrooms in Sakaerat biosphere reverse: *Amanita princeps Corner* & Bas., *A. hemibapha* (Berk. Et Br. Sacc. Subsp. javanica Corner et Bas.), *Russula alboareolata* Hongo, *R. delica* Fr., *R. luteotacta* Rea., *R. aeruginea* Lindbl., *Cantharellus minor* Peck., *Craterellus oderatus* (Schw.) Fr. and *Lentinus polychrous* Lev. This study was correspondingly measured DPPH radical scavenging activity, and total phenolic content which found *Lentinus polychrous* Lev. as one type mushroom with higher antioxidant and *R. luteotacta* Rea. was showed the highest total phenolic contents and ß-carotene [18].

Santhi et al (2016) have studied the antioxidative activities and polyphenoloxidase inhibitory effects of 70% ethanolic extracts from the trimming part of 5 mushroom species. The content of total phenolic compound (TPC), DPPH radical scavenging activity and inhibition of polyphenoloxidase were correspondingly determined that were significant ($p \leq 0.05$) [19].

Yahia et al. (2017) have also studied the identification of phenolic in seventeen species of wild mushrooms in Central Mexico and determination of their antioxidant activity and bioactive compounds. Most species analyzed were edible, but also included nonedible, medicinal, poisonous and toxic specimens that were characterized for water content, color, and total content of phenolic compounds, flavonoids and anthocyanins. The antioxidant capacity was measured by FRAP and DPPH assays and phenolic compounds were identified and quantified by HPLC-mass spectrometry found that all species possessed antioxidant activity and a wide range of phenolic and organic compounds [20].

4. Conclusion

From the test of chemical composition in mushroom specimens of 16 species by the identified analysis of Thin Layer Chromatography (TLC chromatogram) found that every extracted mushroom specimen contained the substance of terpene and steroids but alkaline and tannin were not found in extracted specimens. Moreover, antioxidant activities were found in extracted mushroom specimens of UP_005, UP_007, UP_0014 and UP_0016.

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