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# Phytochemistry and Pharmacological Studies on *Flammulina velutipes*(Curtis)

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***Abstract:***
Researchers around the world has determined and calculating thenutritional values of edible mushroom and also examinedthePharmacological usages. Many edible mushroomscontain bioactive compounds which have various biological activities, such as anti-tumour and anticancer activities and antioxidant properties and anti-bacterial activities.*Flammulinavelutipes*(Curtis),one of the main edible mushrooms on the market was collected from the Yadanabon vegetable market, Mandalay, middle of Myanmar. The phytochemical screening reveals the presence of several groups of secondary metabolites. Antioxidant activity was assessed by DPPH radical scavenging assays and the antimicrobial activities were evaluated by disc diffusion method. Ethanol extract of *F. velutipes* have more antimicrobial action against on Gram-positive and Gram-negative bacteria and also against *Candida albican*than watery extract. The free radicals scavenging activities of extracts of the *F. velutipes*were compared with the positive control ascorbic acid in DPPH free radical scavenging activity method. All extracts have higher antioxidant activity. $IC_{50}$ value of watery extract of *F. velutipes*was found to be as 3.62µg/ml equivalent to that of 1.12µg/ml of ascorbic acid. Antimicrobial activity and antioxidant activity of the mushrooms are due to the presence of phytoconstituents present in it.

*Keywords* **- Phytochemistry, Pharmacological, *Flammulinavelutipes*, DPPH assay**

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## I. INTRODUCTION

A large number of plants worldwide show a strong antioxidant activity and other pharmaceutical activities [1, 2]. Some secondary metabolites are antioxidants compounds that inhibit the autoxidation of lipids and interrupt the autoxidation chain by converting free radicals into more stable species [3].Both cultivated and wild mushrooms contain many secondary metabolites which areantioxidant compounds. Among them polyphenols have some biological actions that include free radical scavenging, metal chelation enzyme modulation activities and inhibition of LDL oxidation [4, 5]. Some compound flavonoid, phenolic acids and anthocyanins are derivatives of polyphenol compoundwhich have highly antioxidant activities [6-8].

The nutritional value and biological activity of mushroom has recognized with the folk medicine, so mushroom have being used in wide spectrum of therapeutic and prophylactic usage. Many medicinal mushrooms areimportant ingredients in Traditional Chinese Medicine, such as*Flammulinavelutipes*(enokitake),*Lentinusedodes*(shiitake), and*Grifolafrondosa*(maitake) [9]. While the scientific community does not known the detail biological properties of mushroom for long time,

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there has been now significant research focused on the sources of mushrooms and also search their therapeutic properties and applications. [10-12].

Mushrooms are worldwide recognition as a functional food as well as a potential source of pharmaceutical sources which may reduce, prevent or treat illnesses.Nowadays, the bioactive compounds isolated from medicinal mushroom are very popular in worldwide beyond their traditional medical usage. [13,14]. *F.velutipes*isalsocommonlyknownasenokitake,velvet shankorgoldenneedlemushroomwintermushroom and its Myanmar name is pin mushroom.

The objective of this study was to evaluate the antioxidant properties of three types of extracts from *Flammulinavelutipes* from middle Myanmar. Their antioxidant activity was evaluated through the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Secondary metabolites, mineral content and antimicrobial activities of *Flammulinavelutipes*were also determined.

## II. MATERIAL AND METHODS

### A. Sampling

*Flammulinavelutipes*was collected from the Yadanabon Vegetable Market, Mandalay, Myanmar. The sample was cut into small pieces and dried in good ventilation place. The dried sample was pulverized and the sample powder was stored in brown bottle for further experiment.

Fig.1 The fresh Enotaki mushroom (*Flammulinavelutipes)*

### B. Determination of Phytoconstituents, Nutritional Values and Mineral

Phytochemical tests were examined according to the standard methods as described by Harbone (1973)[15].

Moisture content of the sample was determined according to American Association of Cereal Chemists (AACC)method 44-15A [16]. (1) g of wasplaced into weighing crucible and placed in muffle finance at100±5ºC for 3 h. The samples were dried and weight again and again until to get the constant weight. After that the moisture contents were calculated.

The ash content was also determined using AACC method 08–01 [17]. (3)g of dried samples was placed in a muffle furnace at 550 ± 10 ºC.They were incinerated until constant weight was obtained. After 7 hour, light gray ash was obtained and ash content was calculated.

Using AOAC method [18], the protein content of the samples was determined by the micro kjedahltechnique. Firstly, the total nitrogen was calculatedand protein was calculated using nitrogen data.

$$N\ (\%) = \frac{volume\ of\ HCl\ x\ N\ x\ 14\ x\ 100}{S\ x\ 1000}$$

Protein content = N x 6.25

where N% is crude nitrogen, N is normality of HCl, 14 is weight of sample, S is the sample weight and 6.25 is conversion factor.

The fat content was determined using Soxhlet apparatus. The percentage crude fat content was calculated using the following equation:

$$\text{Crude fat content (\%)} = \frac{W_2 - W_1}{weight\ of\ sample}\ x\ 100$$

Where: $W_1$ = the weight of empty extraction flask. $W_2$ = the weight of extraction flask after the extraction process with fat.

The mushroom samples (2 g) wasextracted with n-hexane using a Soxhlet apparatus to remove fat. The extracts were dried over anhydrous sodium sulphate, filtered, and concentrated (≤ 30°C) in a rotary evaporator under vacuum. The extracts and fat-free mushroom samples were analysed for their TDF, IDF, and SDF contents by enzymatic and gravimetric methods. Both soluble and insoluble dietary fibres were determined according to the AOAC enzymatic-gravimetric methods. The obtained residue was dialysed at 25°C for 48 hand soluble fibre was subjected to acidic hydrolysis with 1M sulphuric acid at 100±5°C for one and half hour.

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The total carbohydrates were calculated by the following equation [18].

Total CHO = 100 - (moisture% + fat% + protein% + ash% + fiber%).

Total ash was taken for the analysis of mineral contents. The mineral contents were determined by Bench-top Energy Dispersive X-ray Fluorescence (EDXRF).

### C. Determination of antibacterial efficacy Microbial Organisms

The organisms such as gram positive species such as *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) as and gram-negative species such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa*(ATCC 27853) and *Salmonella typhi* (ATCC 33459) which grown in Mueller Hinton agar at 37°C for 24 hours were used for antiracial test determination. Fungal species, unicellular *Candida albicans* (ATCC 60192) which grown in potato dextrose agar at ambient temperature for 72hours was used for antifungal test. The antimicrobial tests weredetermined by disc diffusion methods according toIwalokun et al. [19].

Antimicrobial activities of watery and ethanol extracts of mushroom were determined by the agar well diffusion method[20]. The two extracts were dissolved in 80% ethanol (EtOH) to a final concentration of 10 mg/ml, and it was filtered with 0.45 µm membrane filter. 6 mm in diameter wells were made in the agar plates by sterile cork borer.100 µl of the extracts was loaded into the different wells using micropipette. For these test, positive control, Ciprofloxacin(5µg/well) for bacteria, fluconazole (5µg/well) for fungi and negative control, EtOHwere used. All test plateswere incubated at 37°C, for 24 hours for bacteria and at27°C. 48 hours for fungi. After incubation period, zone ofinhibition was measured in mm (millimetre). All these antimicrobial tests werecarried out in triplicate and their means expressed.

### D. Scavenging Activity on DPPH for Antioxidant Assay

The antioxidant activity of the extracts on the stable radical 1, 1-diphenyl;-2-picrylhydrazyl (DPPH) was determined by the method developed by Feresinet al. (2002)[21]. In this experiment, 1-1 diphenyl -2- picrylhradrazyl (DPPH) powder (stable free radical), Ascorbic acid (standard antioxidant) and Analar grade Ethanol (solvent) were used. The absorbance was determined at 517 nm wavelength. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometer. Firstly, DPPH solution, standard (Ascorbic acid) solution and sample solution were prepared. Radical scavenging activities of Ethanol, Methanol and watery extracts of *Flammulinavelutipes*were also determined.

***1)Preparation of DPPH Solution*:**2.346 mg of DPPH powder was dissolved in 100 ml of ethanol. This solution was thoroughly mixed at room temperature and it was stored in brown colored flask. This solution kept for no longer than 24 hours.

***2)Preparation of Standard Solution*:** 1 mg of ascorbic acid was dissolved in 10 ml of ethanol. This solution was thoroughly mixed at room temperature to obtain 100 µg / ml of standard solution. The five concentrations such as 20, 10, 5, 2.5, 1.25 and 0.625µg/ml were prepared by using two fold dilution methods.

***3)Preparation of Test Sample Solution***: 0.002 g of sample was dissolved in 10 ml ethanol. This solution was thoroughly mixed at room temperature for 15 minutes to obtain 200 µg/ ml of sample solution. The concentrations of sample solutions (20, 10, 5, 2.5, 1.25 and 0.625µg/ml) were also prepared by using two folds dilution method.

After preparation, 1 ml of each ascorbic acid solutions and 3 ml of DPPH solutions were thoroughly mixed and incubated for 15 minutes in the dark room at room. The absorbance of the mixture was measured at 517 nm. In the same way, 1 ml of each sample solutions and 3 ml of DPPH solutions were thoroughly mixed and incubated for 15 minutes in the dark room. The absorbance of the mixture was read at 517 nm.

$$\%\,\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ascorbic acid was used as a positive control in this bioassay. The $IC_{50}$ (concentration providing 50%

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inhibition) was calculated graphically using a calibration curve *vs.* percentage of inhibition.

## III. RESULTS AND DISCUSSION

The phytochemical composition of *Flammulinavelutipes*is presented in Table 1. It reveals the presence of several groups of secondary metabolites.

Table 1: Phytochemical composition of *Flammulinavelutipes*

| Constituents | Reagents | Results |
|---|---|---|
| Alkaloid | Dragendroff's | + |
| Flavonoid | Acetic anhydrous, lead acetate and magnesium turning | + |
| Phenolic | 10% $FeCl_3$ | + |
| Polyphenol | 1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$ | + |
| Steroid | Acetic anhydride and conc:$H_2SO_4$, $CHCl_3$ | + |
| Terpene | $CHCl_3$ and conc:$H_2SO_4$ | + |
| Glycoside | 10% lead acetate | + |
| Carbohydrate | Fehling' (A) and (B) solution | + |
| Tannin | 10% $FeCl_3$, dil $H_2SO_4$ | + |
| Saponin | Water | + |
| Lipophilic group | 0.5% M KOH solution | + |

+ present : - absent

Several nutritional parameters were measured for bothfresh and dried mushroom sample.

Table 2:Nutritional value of fresh and dried sample (g/100g)

| Enokie mushroom | Nutritional value | | | | | |
|---|---|---|---|---|---|---|
| | moisture | Ash | protein | fat | fibre | CHO |
| Fresh | 87.5 ± 0.35 | 1.13 ± 0.03 | 2.6 ± 0.18 | 0.54 ± 0.05 | 3.0 ± 0.11 | 5.24 ± 0.40 |
| Dry | - | 9.00 ± 0.48 | 20.26 ± 1.45 | 4.50 ± 0.29 | 23.31 + 1.3 | 42.83 ± 2.54 |

**Values are the average of triplicate experiments and represented as mean ± Standard deviation*

The moisture content of fresh *F.velutipes*was found about 87.5±0.35 %. Moisture contents of the mushroom are high, indicating that mushrooms are highly perishable. Breene (1990)[22] and Çokuner and Özdemir (2000)[23] express protein contents of mushrooms range from 19 to39 g in 100g dried matter. It was found that *F.velutipes* contains the protein values as 2.6±0.18 (g/100g fresh matter) and 20.26 ± 1.45 (g/100g dried matter). Fat content was 4.50 ± 0.29 in 100g in drymatter of *F.velutipes*. This result isconformable with Shin et al. (2007)[24]. In some literature, the carbohydratevalues of mushrooms was estimated as 40 ~ 45 g in 100g dry matters [25]. In this research work, carbohydrate value was found to be42.83 ± 2.54 (g/100g dried matter) which value is almost similar the study made by Watanabe et al. (1994)[25].

Mushrooms are also rich in mineral contents. The total ash content found in *F.velutipes* was 1.13 g/100g fresh. In case of dry mushrooms it was 9.00 g/100g dry matter.The contents of some important minerals in 100 g of dried *Flammulinavelutipes*contained K (39.10%), P (4.65%), Mg (1.23 %), Ca (0.14%), Cl(0.55%), Fe (0.18%), Si (0.16%), Al (0.14%) and Zn (0.04%). Toxic heavy metal such as As and Pb are not detected.

The present study has revealed the antimicrobial activity of mushroom extract. The mushroom extracts used in this study were found to exhibit various degrees of antimicrobial effects against thetested microorganisms. The zone of inhibition exhibitedmore than 10 mm was considered as highly activefor extracts.

Table 3: Preliminary antimicrobial testing of mushroomextracts through determination of zone of inhibition(mm ± SD)*

| Organisms | EtOH | Aqueous | standard drug |
|---|---|---|---|
| *Staphylococcus aureus* | 13.5 ± 0.1 | - | 25 ± 0.2 |
| *Bacillus subtilis* | 14.1 ± 0.2 | 9.0± 0.2 | 24 ± 0.1 |
| *Escherichia coli* | 11.5 ± 0.1 | 11.0 ± 0.1 | 24 ± 0.3 |
| *Pseudomonas aeruginosa* | 7.5 ± 0.2 | 7.0± 0.2 | 20 ± 0.2 |
| *Salmonella typhi* | 15.5± 0.1 | 7.0± 0.1 | 20 ± 0.2 |
| *Candida albican* | 12.5 ± 0.1 | 7.0 ± 0.1 | 25 ± 0.1 |

*The diameters of zone of inhibition were expressed in millimeter (mm) asmean ± standard deviation (SD).

The ethanol extract shows high activity on all selected microorganisms except*P. aeruginosa*. But the watery extract has high activity on only *E. coli* and medium activity on *B. subtlis, P. aeruginosa,*

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*salmonella typhi* and *C. albican*. Watery extract has no activity on *S. aureus*.

In the DPPH assay, the antioxidants are able to reducethe stable DPPH radical (purple) to the non-radical formDPPH-H (pale yellow or colorless). The DPPH scavenging activities ofantioxidants are attributed to their hydrogen donatingabilities. All the mushroom extracts showed the antioxidant activity. Watery extract has high antioxidant activity than methanol and ethanol extract.

Table 4: Free radical scavenging activity ($IC_{50}$µg/ml)

| Extracts | % inhibition (mean ± SD) in different concentrations (µg/mL) | | | | | | $IC_{50}$ µg/mL |
|---|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | 20 | |
| Ethanol | 17.77 ±1.22 | 32.15 ± 1.04 | 45.34 ± 0.04 | 53.22 ± 0.32 | 61.45 ± 0.11 | 69.01 ± 0.10 | 3.84 |
| Methanol | 13.98 ± 2.35 | 17.06 ± 1.34 | 41.00 ± 0.84 | 53.08 ± 0.84 | 57.35 ± 0.84 | 61.61 ± 1.10 | 4.49 |
| Aqueous | 14.95 ± 1.15 | 26.63 ± 2.31 | 43.21 ± 0.96 | 57.34 ± 2.69 | 61.96 ± 1.54 | 64.13 ± 1.15 | 3.62 |
| Ascorbic acid | 14.04 ± 2.09 | 54.83 ± 2.48 | 72.44 ± 3.83 | 81.13 ± 1.47 | 87.40 ± 2.23 | 91.21 ± 0.48 | 1.17 |

The $IC_{50}$ values were found to be 3.62 µg/ml for Ascorbic acid, 3.62µg/ml for watery extract, 3.84µg/ml for ethanol extract and 4.49µg/ml for methanol extract, respectively. It means watery extract of sample captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in $IC_{50}$ value.

## IV. CONCLUSIONS

*Flammulinavelutipes* mushroom studied were found to be a good source of protein, fibre andminerals. Both extracts of *Flammulinavelutipes*show inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans.* Watery extract has low activity than ethanol extract. From this study, the watery extract of *Flammulinavelutipes*was found to be higher antioxidant activity, which may be due to the presence of phenols and flavonoids in this mushroom. According to the nutritional value, mineral contents, antimicrobial activity and the electron scavenging activity of Enokitake mushroom (*Flammulinavelutipes)*indicate that it can be used as food as well as medicine.

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## REFERENCES

[1] M. C. Baratto, M.Tattini, C. Galardi, P. Pinelli, A. Romani, F. Visiolid, et al., "Antioxidant activity of Galloylquinic derivatives isolated from *Pistacialentiscus* leaves", *Free Radical Research*. 37(**4**): p.405- 412, 2003.

[2] V. Katalynic , M. Milos, T. Kulisic, M. Jukic, "Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols", *Food Chemistry*, 94: p.550–557, 2006.

[3] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "Free radicals, antioxidants and functional foods : Impact on human health". *Pharmacognosy Review*,4(**8**), 118–126, 2010.

[4] PL Teissedre , N Landrault, "Wine phenolics: contribution to dietary intake and bioavailability". *Food Res Int* .33: 461-467, 2000.

[5] R. Rodrigo, C. Bosco, "Oxidative stress and protective effects of polyphenols: comparative studies in human and rodent kidney". A review. *Comp BiochemPhysiol Part C ToxicolPharmacol,* 142: 317-327, 2006.

[6] O. Isıldak, I. Turkekul , M. Elmastaş, M. Tüzen, "Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region", *Turkey. Food Chem* 86: 547-552, 2004.

[7] I. Türkekul , M. Elmastaş, M. Tüzen M, "Determination of iron, copper, manganese, zinc, lead, and cadmium in mushroom samples from Tokat, Turkey". *Food Chem* 84: 389-392, 2004.

[8] [8] M. Elmastas, O. Isıldak , I. Turkekul, N. Temur, "Determination of antioxidant activity and compounds in wild edible mushrooms". *J Food Compost Anal* 20: 337-345, 2007.

[9] R. Sullivan, J. E. Smith, and N. J. Rowan, "Medicinal mushroomsand cancer therapy: translating a traditional practice into Westernmedicine". Perspect. *Biol. Med.*49, 159–170, 2006.

[10] A. B. Encarnacion, F. Fagutao, O. Jintasataporn, W. wattanamateekul, I. Hirono, and T. Ohshima, "Application of ergothioneine-rich extract from an edible mushroom *Flammulinavelutipes* for melanosis prevention in shrimp, *Penaeusmonodon* and *Litopenaeusvannamei*', *Food Res. Int.*45,232–237, 2012.

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[11] P. Kalaˇc, "A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms", *J. Sci. Food Agr*.93, 209–218, 2013.
[12] A. A. Soares, A. B. de Sa-Nakanishi, A. Bracht, S. M. da Costa, E. A. Koehnlein, C. G. De Souza, et al., "Hepatoprotective effects of mushrooms". *Molecules*, 18, 7609–7630, 2013.
[13] H. Wang, T. O. Khor, L. Shu, Z. Su, F. Fuente, J. -H. Lee, et al. "Plants against cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability", *Anticancer Agents Med. Chem*.12, 1281, 2012 a
[14] Q. Liu, G. Tian, H.Yan, X. Geng, Q. Cao,H. Wang, et al. "Characterization of polysaccharides with antioxidant and hepatoprotective activities from the wild edible mushroom *RussulavinosaLindblad*".*J. Agric.Food Chem*.62, 8858–8866, 2014.
[15] J. B. Harborne, "Phytochemical Methods". London. Chapman and Hall Ltd. pp: 49-188, 1973.
[16] Technical, A. Ash-Basic Method. AACC Int. Approv. Methods **2009**.
[17] Technical, A. Moisture-Air-Oven Methods. AACC Int. Approv. Methods **2009**.
[18] A.O.A.C. Association of Official Analytical Chemists Official methods of analysis, 11th edition. Washington, D. C., 2000.
[19] B. A. Iwalokun , A. Ogunledun ,D. O. Ogbolu, S. B. Bamiro, J. Jimi-Omojola, "In vitro antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria". *J Med Food*. 7:327–33, 2004.
[20] V. O, Oyetayo, C. H. Dong , Y. J. Yao. "Antioxidant and antimicrobial properties of aqueous extract from *Dictyophoraindusiata". Open MycoJ*. ;3:20–6, 2009.
[21] G. E. Feresin, A. Tapia, G.R. Angel, C. Delporte, N. B. Erazo, G. Schmeda-Hirschmann, "Free radical scavengers, anti-inflammatory and analgesic activity of Acaenamagellanica". *J Pharm Pharmacol*. ;54:835–44, 2002.
[22] W. M.Breene, "Nutritional and medicinal value of specialtymushrooms". *J. Food Protect*. 53:883-894, 1990.
[23] Y. Ço_kuner and Y. Özdemir, "Acid and EDTA blanching effects on the essential element content of mushrooms (*Agaricusbisporus*)". *J. Sci. Food Agric*. 80:2074-2076, 2000.
[24] C. K. Shin, C. F, Yee, J. S. Lee and M. Atong, "Nutri-tional properties of some edible wild mushrooms in Sabah". *J.Appl. Sci*. 7:2216-2221, 2007.
[25] T. Watanabe, N. Tsuehihasi, Y. Takai, K. Tanaka, and A. Suziki, A. "Effects of ozone exposure during cultivation of oystermushroom (Pleurotusostreatus) on chemical components of the fruit bodies". *J. Jpn. Soc. Food Sci. Technol*. 41:705-708, 1994.

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