Phytochemical analysis of *Schizophyllum commune* Fr. and *Microporus xanthopus* (Fr.) Kuntze from Phulchowki, central Nepal

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Abstract: Mushrooms are widely known for their therapeutic properties, which can be attributed to the secondary metabolites they produce. This study aims to evaluate different phytochemical constituents of two saprophytic mushrooms, namely *Schizophyllum commune* Fr and *Microporus xanthopus* (Fr.) Kuntze, collected from forest around Phulchowki, Lalitpur, Nepal. The total phenolic, flavonoid, vitamin C, β-carotene, and lycopene contents of *S. commune* were found higher than *M. xanthopus* (143.21 ± 0.003 vs. 108.45 ± 0.112 mg GAE/100 g; 91.55 ± 0.121 vs. 49.72 ± 0.073 mg QAE/100 g; 26.67 ± 0.015 vs. 3.15 ± 0.170 mg AA/100 g; 0.036 ± 0.001 vs. 0.013 ± 0.002, and 0.026 ± 0.002 vs. 0.01 ± 0.004 mg carotenoid/g; respectively) in the methanolic extracts. This study suggests that these mushrooms might have some medicinal values.

Keywords: *S. commune*; *M. xanthopus*; Phenols; Flavonoids.

Introduction

Mushrooms are important natural sources of nutrition and could possess medicinal values[1]. They are rich in fiber, low in calories and cholesterol, and are packed with essential vitamins and minerals[2,3]. They also have therapeutic properties, which have been related to the presence of various bioactive constituents present in the fruiting bodies and mycelium of mushrooms[4–6].

Secondary metabolites are biologically active compounds produced by organisms to defend themselves against harsh environmental circumstances or predatory threats. However, they are not essential to their normal growth and reproduction[7,8]. Medicinal mushrooms produce several types of secondary metabolites, like terpenoids, organic acids, alkaloids, lactones, polyphenolic chemicals, vitamins, nucleotide analogues, sterols, and metal chelating agents[5,9]. These compounds make them valuable therapeutic agents in the treatment of infections and illnesses, and could be an alternative for antibiotics in fight against pathogens[10,11].

Schizophyllum commune Fr. and *Microporus xanthopus* (Fr.) Kuntze are saprophytic macrofungi that belongs to family Schizophyllaceae and Polyporaceae, respectively. They are commonly found in forests with dead wood abundance[12,13]. *S. commune* is characterized by split gill and is therefore also called split gill mushroom[14]. It is consumed as food, and medicine in some counties, e.g. India, Mexico, Malaysia and Indonesia[15,16]. *M. xanthopus*, also known as yellow-footed polypore, are funnel shaped with concentric brown, and creamy shades on the inner surface[17]. These two mushrooms have been widely used as food, tinder, and commodities[12.]. They contain important phytochemical components such as flavonoids, alkaloids, phenols, triterpenes, and organic acids[17–19]. They also possess biological properties including antibacterial[17], anti-tumor[20], anti-fungal[19], anti-inflammatory[21], and cytotoxic activity[22].

Nepal is home to a large number of mycoflora. Although much work has been done to gather and identify Nepalese

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mushrooms but the phytochemical analysis for medicinal properties has been rarely explored. The main objective of this study was to determine the phytochemical composition of two wild mushrooms (*M. xanthopus* and *S. commune*) for further application.

Methods

1. Sample collection and preparation

Fresh fruiting bodies of two mushroom samples were collected from rotting tree branches in Phulchowki, Godawari, Lalitpur (central hill area, 27.5711°N, 85.4056° E), Nepal, in October 2020. The mushrooms were labeled as M1 and M2, respectively. The samples were sealed in ziploc bags and transported to the Biological Resources Unit Laboratory, Nepal Academy of Science and Technology (NAST) within 2 hours. The collected mushrooms were identified by Dr. Jay Kant Raut and compared with reference strains. M1 appeared to have radial, central split gills consisting of folds, and was identified as *S. commune*[23]. M2 sample contained thin funnel shaped caps which were concentrically zoned in different shades of brown, and was confirmed as *M. xanthopus*[24]. The mushrooms were cured in shade for a week. Samples were thoroughly examined for any bacterial or fungal contamination. A grinder was used to convert the mushroom into fine powder. The powdered samples were stored in airtight containers at 4°C until further evaluation.

2. Phytochemical analysis

2.1. Mushroom extract preparation

The phytochemical constituents in the samples were evaluated using a methanolic extraction method as described by Al-Harrasi et al[25]. In brief, 1 gram of each dried, and powdered mushroom samples was weighed separately, and 20 mL methanol was added to the sample. The mixture was shaken at 100 revolutions per minute (rpm) at 37°C for 24 hours in a shaking incubator. This was followed by filtration of the mixture through Whatman No. 1 filter paper, and the filtrates obtained were kept at 4°C. Fresh methanol (20 mL) was added to the residue, and the mixture was then placed in a shaking incubator at 100 rpm in 37°C for 24 hours. Next, the mixture was filtered using a Whatman No. 1 filter paper. All the extracts from first and second filtrations were combined. The samples were evaporated to dryness in a rotary evaporator at 40°C and were stored at 4°C for further investigations[26].

2.2. Total phenolic content

Total phenolic content in the mushroom samples was estimated using a modified Folin-Ciocalteu assay[27]. Briefly, 150 µL Folin and Ciocalteu's phenol reagent was thoroughly mixed with 50 µL of the methanolic extract. After 3 min, 150 µL of a saturated sodium carbonate (Na_2CO_3) solution was added to the mixture, and the volume was adjusted to 1500 µL with milli-Q water. The reaction was placed in dark for 90 min. Next, 200 µL of the solution was transferred to an optically clear 96 well plate, and the absorbance was measured at 725 nm using ELISA plate reader (ThermoFisher Scientific, USA). Gallic acid at different concentrations was used as a standard (25-500 µg/mL; $y = 0.001x + 0.052$; $R^2 = 0.999$). The result was expressed as mg of gallic acid equivalent (GAE) per 100 g of extract.

2.3. Flavonoid content

Total flavonoid content was determined using modified aluminum chloride ($AlCl_3$) method[28]. Firstly, 100 µL of 2% $AlCl_3.H_2O$ solution was added to same volume of mushroom extract. The mixture was incubated in the dark for an hour, and absorbance was recorded. Quercetin at different concentrations was used to calculate the standard curve (25-250 µg/mL; $y = 0.013x + 0.013$; $R^2 = 0.995$). The result was expressed as mg of quercetin equivalent (QAE) per 100 g of extract.

2.4. Vitamin C content

Vitamin C content was measured employing modified Klein and Perry method[29]. For this, 50 mg dry methanolic extract of the mushroom samples was extracted in 5 mL of 1% meta-phosphoric acid for 45 min at room temperature. The solution was filtered through Whatman No. 4 filter paper. To 100 µL of each sample, 900 µL of 2,6-dichlorophenolindophenol (DCPIP) was added and mixed properly. 200 µL of the reaction mixture was added to 96

well plates, and the absorbance was measured within 30 min at 515 nm against a blank. Ascorbic acid was used to calculate the standard curve (25-100 µg/mL; y = 0.001x - 0.002; R^2 = 0.0994). The result was expressed as mg of ascorbic acid (AA)/100 g of extract.

2.5. β-carotene and lycopene content

β-carotene and lycopene contents were measured using modified Nagata and Yamashita method[30].

After weighing 100 mg dried methanolic extract, 10 mL of acetone-hexane mixture (4:6) was added for 1 min. The mixture was then agitated and filtered through Whatman No. 4 filter paper. Next, 200 µL of the sample was transferred to the ELISA plate and absorbance was measured at 453, 505, and 663 nm, respectively. β-carotene and lycopene concentrations were calculated according to the following equations: lycopene (mg/100 mL) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

β-carotene (mg/100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

The results were expressed as µg of carotenoid/g of the sample.

Statistical analysis

All measurements were obtained in triplicate. Data were analyzed using Microsoft Excel and expressed as mean ± standard deviation.

Figure: 1. *S. commune* (M1) and *M. xanthopus* (M2) collected from Phulchowki, Godawari.

Results and discussion

Mushroom collection

Figure 1 shows the mushroom collected from forest of Phulchowki, Lalitpur. They were saprophytic as they were found growing on rotten tree branches. The phytochemicals estimated in *S. commune* and *M. xanthopus* included phenols, flavonoids, vitamin C, lycopene, and β-carotene (Table 1).

Table : 1. Phenols, flavonoids, Vitamin C, β-carotene, and lycopene contents (mean ± SD) in methanolic extract of mushroom samples.

Parameters	Unit	Concentration of the phytochemicals	
		M1 (*S. commune*)	**M2 (*M. xanthopus*)**
Phenol	(mg GAE/100g)	143.21 ± 0.003	108.45 ± 0.112
Flavonoid	(mg QAE/100g)	91.55 ± 0.121	49.72 ± 0.073
Vitamin C	(mg AA/100g)	26.67 ± 0.015	3.15 ± 0.170
β-carotene	(mg carotenoids/g)	0.036 ± 0.001	0.013 ± 0.002
Lycopene	(mg carotenoids/g)	0.026 ± 0.002	0.01 ± 0.004

The phenolic compounds like phenol and flavonoid are known to exhibit potent anti-cancer, anti-inflammatory, antimicrobial activities as well as combat various diseases associated with oxidative stress[31]. In our study, the total phenol contents from *S. commune* and *M. xanthopus* were found to be 143.21 ± 0.003 and 108.45 ± 0.112 mg GAE/100g, respectively. These phenolic content was lower than the previous studies for the same species (*S. commune*: 172 ± 0.05 mg GAE/100g[32], and *M. xanthopus*: 3882.50 ± 348 mg GAE/100g[33]). The concentration for *Microsporus* spp. was similar to *Polysporus gilvus* but was lower than other similar species[34].

Flavonoids are the most significant phenolics having various biological and chemical activities in addition to radical scavenging property[35]. In this study, the total flavonoid contents from M1 and M2 were 91.55 ± 0.121 and 49.72 ± 0.073 mg QAE/100g, respectively which is less than the study conducted by Juliette-Ornely et al[33] and Tangjitjaroenkun, and Tangchitcharoenkhul[36]. The flavonoid content of *Polyporus umbellatus* and *Sparassis nemecii* was lower as compared to the flavonoid level observed in our study (4.3-4.6 mg

QAE/g dw)[37].

Vitamin C is water-soluble nutrients that are required for numerous biochemical and physiological activities in the body. It is essential for bone growth, wound healing, gum health maintenance, vitamin B and folic acid activation[38,39]. In our study, *S. commune* and *M. xanthopus* contained vitamin C in considerable amount.

Fungi are guarded against oxidative stress and non-ionizing radiation like UV light by carotenoids[40]. β-carotene and lycopene are carotenoids, which are natural pigments present in mushrooms, and can exert antioxidant and anti-inflammatory properties. β-carotene is the precursor for the production of vitamin A[41]. In our study, β-carotene level in *S. commune* and *M. xanthopus* were 0.036 ± 0.001 mg/g and 0.026 ± 0.002 mg/g, respectively.

These secondary metabolites produced by mushrooms are useful in medicinal chemistry. *S. commune* and *M. xanthopus* have antibacterial[17], anti-inflammatory[21], cytotoxic[22], and antioxidative properties[42]. These phytochemicals could be a potent source for creating novel antibiotics.

Conclusion

The higher levels of phenolic compounds, flavonoids, vitamin C, β-carotene, and lycopene in *S. commune* and *M. xanthopus* suggest their potential medicinal properties. There is a need for additional investigation on bioactive compounds present in these mushrooms for therapeutic purposes.

Author's contribution

PD and LRB designed the study. PD conducted lab experiments and analyzed the data. MS and CK interpreted the data and drafted the original manuscript. JKR and LRB supervised the research work, revised, and edited the manuscript. All authors revised and approved the final manuscript.

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Conflicts of interests

The authors declare no conflicts of interest regarding the research work.

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