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RESEARCH ARTICLE

Harnessing the nutraceutical and antioxidant potential of the sporocarp of medicinal mushrooms, *Schizophyllum commune* Fr.

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Abstract

The split gill mushroom, *Schizophyllum commune* Fr., naturally occurs on decaying wood during the rainy season and is consumed as a food source in many parts of North-East India due to its medicinal properties. In the present study, an isolate of *S. commune* was analysed for its proximate composition and nutraceutical potential to support the development of biofortified products. The proximate composition analysis of dried and fresh mushrooms of *S. commune* revealed the following results: carbohydrate content (74.00% and 30.51%, respectively), calorific value (358.00 and 148.84 Kcal/100g, respectively), crude fiber (3.08% and 1.20% respectively), crude protein content (13.70% and 6.18%, respectively) and total antioxidant activity (1267.00 µg/g and 1090.76 µg/g, respectively). Glycemic potential assessments indicated that *S. commune* exhibited a low glycemic index of 10.33 and total starch digestibility of 7.65%. The nutraceutical analysis of dried *S. commune* powder in different solvent extracts (methanol, ethanol and ethyl acetate) demonstrated that methanolic extracts recorded the highest DPPH inhibition percentage (89.60%), reducing power (37.20%) and phenolic content (0.230 ± 0.005 mg/mL). Methanolic extracts also exhibited higher flavonoid content (0.455 ± 0.003mg/mL). Ethanolic extracts were found to have the highest total tannin (0.250 ± 0.001mg/mL) and saponin content (0.303 ± 0.001mg/mL). A herbal infusion formulated with *S. commune* dried powder, dried basil leaf powder and dried ginger powder demonstrated high antioxidant and phytochemical properties. Sensory evaluation of two treatments of the herbal infusion indicated good overall acceptability, highlighting its potential as a commercially viable fortified product with significant antioxidant properties.

Keywords

antioxidant properties; biofortified product; herbal infusion; nutraceutical properties; *Schizophyllum commune*

Introduction

Schizophyllum commune Fr., a white-rot basidiomycetous fungus, is known for its high medicinal value and possesses a natural habitat on the trunk of dead trees during the rainy season in both temperate and subtropical regions. The split gill mushroom, *S. commune*, was first named by Swedish mycologist Elias Magnus Fries in 1815. It is referred to as a white-rot fungus or split gill mushroom. The name *S. commune* is derived from the Greek term *Schiza*, meaning "split," and *commune*, signifying "common" (1). The fruiting body of *S. commune* is a small, flabelliform (fan-shaped), white cap with hairs and a rudimentary stipe. This mushroom can be

cultivated on locally available agricultural wastes such as paddy straw, wheat straw and sawdust, making it relatively easier to grow compared to other medicinal mushrooms (2, 3).

S. commune is highly regarded as a nutritional food and medicinal resources in several nations, including Korea, Malaysia, China, Thailand and Vietnam, owing to its significant medicinal properties. In India, particularly in the northeastern regions, this mushroom is widely consumed for its nutraceutical benefits. It is especially favoured among the Mizo community due to its antimicrobial, anti-cancerous, antidiabetic and antioxidant activities against many human diseases (4). However, a standardized cultivation technology for *S. commune* is yet to be developed. The existing literature indicates that the mushroom is cultivated using substrates like straw and sawdust, but the yields remain low. *S. commune* is a potent lignin-degrading fungus with advanced lignocellulolytic machinery, making it suitable for industrial applications (5). Therefore, its cultivation could be optimized on lignin-rich substrates, as the degradation of plant lignocellulose by *S. commune* is facilitated through a hydroxyl radical-mediated mechanism in conjunction with a synergistic system of various polysaccharide-degrading enzymes (6).

The cultivation of *S. commune* is still in its preliminary stage in South Indian geographical conditions. Standardizing its cultivation methodology will enable oyster mushroom growers to incorporate the cultivation of this sub-tropical medicinal mushroom *S. commune* into their existing cropping systems.

S. commune has extensive pharmaceutical and nutraceutical applications that remain underutilized. The exopolysaccharides produced by the mushroom have significant pharmaceutical potential. For instance, the polysaccharide Schizophyllan *S. commune* is widely recognized for its high medicinal value (7). Phenolic compounds such as phenyl benzoate ($C_{13}H_{10}O_2$) and 4-(phenyl methoxy) phenol ($C_{13}H_{12}O_2$) identified through GC-MS analysis from ethanolic extract of *S. commune*, demonstrate potential as bioactive compound (8). Additionally, extracellular melanin produced by *S. commune* exhibits antibacterial, antifungal and anti-cell proliferation activity against human epidermoid larynx carcinoma cell lines *S. commune* (9). Different solvent extracts of *S. commune* also display antidiabetic activity (10). Bioactive molecules, including polysaccharides, triterpenoids, low-molecular-weight proteins, glycoproteins and immunomodulating compounds, are widely employed in anticancer treatment (11). Furthermore, bioactive compounds, specifically Schizostatin derived from the *S. commune*, exhibit antimicrobial activity against plant pathogens of pepper (12).

To enhance yield with maximize bio efficacy, this study aimed to refine and standardize the cultivation methods of *S. commune* using readily available substrates like paddy straw. Additionally, the study sought to investigate its nutrients and nutraceutical properties for the development of fortified food products. In this context, the research was designed to screen and explore the nutraceutical and antimicrobial properties of *S. commune* and to develop food products incorporating *S. commune* as a key ingredient.

Materials and Methods

Estimation of proximate composition of S. commune (Sch.2)

The nutrients and proximate composition of *S. commune* were analysed using fresh and dried basidiocarps at the Centre for Post-Harvest Technology, TNAU, Coimbatore. The analysed parameters included carbohydrates (%), protein (%), crude fiber (%), calorific value (Kcal/100g), total phenols (mg/100g), total antioxidant activity (µg/g), water activity (a_w) and mineral content such as potassium (ppm), sodium (ppm), zinc (ppm). Additionally, the color value (L*, a*, b and dE) of the mushroom were evaluated. Each proximate composition analysis was performed (13).

Investigation of nutraceutical properties of S. commune (Sch.2)

S. commune exhibits a variety of bioactive properties, including antioxidant, antimicrobial, antidiabetic and anti-tumor activities. To investigate its nutraceuticals potential, three different solvents *viz.,* methanol, ethanol and ethyl acetate were employed to extract its bioactive compounds. The extraction was performed at a concentration of 100µg/ml for analysis.

For the extraction process, 100 grams of harvested mushrooms were dried in an oven at 45-50°C until the moisture content reached 10%. The dried mushrooms were then ground into a fine powder using a pestle and mortar. Ten grams of this powder were dissolved in 100 mL of methanol, ethanol and ethyl acetate individually. The mixture was agitated at 150 rpm in a shaker and subsequently filtered using Whatman No. 1 filter paper. The resulting filtrates were evaporated to dryness at 40°C and the dried extracts were reconstituted in their respective solvents for further nutraceutical analysis (4, 14-15).

Antioxidant assays

The antioxidant potential of the *S. commune* isolate *Sch.*2 was evaluated using the following assays: such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power and total phenol determination were performed. All experiments were performed with five replicates to ensure reliability of the results.

Assay of DPPH radical scavenging activity

To assess DPPH radical scavenging activity, 100 µL of the sample was mixed with 3 mL of ethanol and filtered. From the filtrate, 10 µL was diluted with 90 µL of ethanol to make a 1 mL solution. To this, 1 mL of DPPH reagent prepared in ethanol was added. The mixture was shaken thoroughly and kept in the dark for 20 minutes. The absorbance was measured at 517 nm using a UV -VIS spectrophotometer. The percentage of DPPH inhibition was calculated using the following formula (16).

$$\%\text{PPH inhibition} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

Assay of reducing power

The reducing power of the samples was analyzed (17). A solution was prepared by mixing 2.5 mL of the respective solvent extracts, 2.5 mL of 200 mM sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of 10% trichloroacetic acid. It was then centrifuged at 3000 rpm for

10 minutes. After centrifugation, 5 mL of the upper layer was mixed with 5 mL of ultra-pure water and 1 mL of 0.1% ferric chloride solution. The absorbance was recorded at 700 nm. The percentage reducing power was determined using the following formula:

$$\%\text{Reducing power} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

Determination of total phenolic content

To determine the total phenolic content, 50 µL of solvent extracts at a concentration of 100 µg/µL was mixed with 50 µL of Folin-Ciocalteu phenol reagent. After 3 minutes, 50 µL of a 20% saturated sodium carbonate solution was added and the volume was adjusted to 300 µL with distilled water. The solution was kept in the dark for 90 minutes and the absorbance was measured at 725 nm using a UV-VIS spectrophotometer (18). The total phenolic antioxidant capacity was estimated using the following formula:

$$\%\text{Total phenolic antioxidant capacity} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} \times 100$$

Phytochemical analysis of solvent extraction of S. commune

The phytochemical analysis of *S. commune* isolate Sch.2 included assays for total saponin, flavonoid and tannin content. All experiments were conducted in five replications.

Estimation of total saponin

The vanillin-sulphuric acid assay method was used to estimate total saponin. 50 µl of different solvent extract solutions were added to separate test tubes, followed by the addition of 0.25 ml of vanillin reagent (8%, in 99.9% ethanol). The test tubes were placed in an ice-cold water bath, 2.5 ml of 72% sulphuric acid was slowly added along the inner wall of the tubes. After mixing, the tubes were allowed to stand for 3 minutes. Subsequently, the tubes were placed in a water bath for 10 minutes and then cooled in an ice bath. A standard curve was developed by measuring the absorbance at 544 nm against a reagent blank using a spectrophotometer. Quillaja saponin (Sigma-Aldrich) was used as the standard (19).

Estimation of total flavonoid content

The total flavonoid content was estimated by taking, 0.2 mL of the sample into test tubes and diluting the volume to 3 mL with distilled water. Subsequently, 0.2 mL of 10% aluminum chloride and 0.2 mL of 1 M potassium acetate were added. The reaction mixture was kept at room temperature for 30 minutes. The absorbance was measured at 420 nm using a spectrophotometer. The flavonoid content was calculated by extrapolating a calibration curve created using a genistein solution prepared in hot methanol. The results were expressed as milligrams per millilitre (20).

Estimation of total tannin

The total tannin content was calculated following the procedure (21). Using the Folin-Ciocalteu method, a 10 mL volumetric flask was filled with distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% sodium carbonate solution. The mixture was diluted to 10 mL with distilled water, thoroughly shaken and allowed to stand at room temperature for 30 minutes. Tannic acid reference standard solutions (20, 40, 60, 80 and 100 µg/mL) were prepared similarly. The absorbance of the test and standard solutions was measured at 700 nm using a UV-Visible spectrophotometer against a blank.

Anti-diabetic assay

The glycemic index of *S. commune* mushroom powder was determined (22). One gram of the sample was ground into a paste with 2 mL of water and the volume was increased to 12 mL. To this, 4 mL of alpha-amylase (250 U/mL) was added and the pH was adjusted to 2-2.3. The mixture was shaken for 20 seconds and then left undisturbed for 5 minutes. Subsequently, 3 mL of protease (2.5 U/mL) was added and incubated at 37°C in a shaker for 30 minutes. After incubation, the pH was adjusted to 6.0 using 2N NaOH, followed by the addition of 2 mL of pancreatin (2 mg/mL).

Next, 2 mL of alpha-glucosidase (28 U/mL) was added and the mixture was shaken at 37°C for 4 hours. During this period, 1 mL aliquots were taken every 30 minutes, centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. A 30 µL aliquot of the supernatant was mixed with 1 mL of DNS reagent and placed in a boiling water bath for 10 minutes. Afterward, the volume was made up to 7 mL with distilled water. A yellow color developed and the absorbance was measured at 520 nm. The total starch digestibility and glycemic index were calculated using the obtained absorbance values and the following formula:

$$\text{Total Starch Digestibility} = \frac{\text{Sample OD value} + 0.003}{0.001}$$

$$\text{Glycemic index} = \frac{\text{Total starch digestibility}}{\text{Total sugar content}} \times 100$$

Development of herbal infusion of S. commune

Considering the nutraceutical properties and antidiabetic assay of *S. commune* isolate *Sch.*2 in different solvent extracts, it was observed that *S. commune* possesses potential for use as a biofortified or functional food. Based on these findings, the development of a herbal infusion was planned, following methodologies reported in the literature on moringa herbal infusion. The preparation of the herbal infusion was conducted (23).

For the preparation of dried basil leaf powder, 1 kg of fresh basil leaves was purchased from the market. The leaves were picked, washed with clean water and drained using a nylon net. After excess water was removed, the leaves were dried in a cabinet dryer at 55°C, coarsely ground and stored in airtight polythene bags. A similar process was followed for ginger: the outer skin was peeled off, the ginger was washed, cut into small pieces, dried in a cabinet dryer and ground into a coarse powder. The herbal infusion was prepared in different combinations such as (T1 - *S. commune* dried powder alone 100:0:0), (T2 - *S. commune* dried powder+ dried basil leaves powder+ dried ginger powder 50:25:25), (T3 - Dried basil leaves powder+ dried ginger powder 50:50).

Each mixture was filled into drawstring tea filter bags (size 5.5 × 7 cm) with 0.5 g of table salt added to each bag. Each bag contained 2 g of the respective ingredient combinations for the three treatments. To prepare the herbal infusion, the tea

bags were steeped in 100 mL of hot water at 70°C and left for approximately 2 minutes for straining. The tea bag was then removed and the herbal infusion was ready for consumption and subsequent analysis.

Sensory evaluation of herbal infusion and analysis of antioxidants and phytochemicals

To validate the developed product, a sensory evaluation was conducted by experts from the department. The evaluation assessed all three combinations of herbal infusions (T_1 *S. commune* dried powder alone; T_2 *S. commune* dried powder+ dried basil leaves powder+ dried ginger powder; T_3Dried basil leaves powder+ dried ginger powder). The scoring scale ranged from 9 to 1, where 9 indicated “Like Extremely” and 1 indicated “Dislike Extremely”.

The sensory evaluation focused on the following parameters: appearance, color, flavor, consistency, taste and overall acceptability of the product. This systematic assessment ensured a comprehensive evaluation of the sensory attributes of the developed herbal infusions.

Statistical Analysis

Statistical software was utilized to analyze the experimental design, including Completely Randomized Design (CRD) and Randomized Block Design (RBD) (24). For the sensory evaluation of the biofortified product, the assessment was conducted with faculty, colleagues and staff members from the Department of Plant Pathology, TNAU, Coimbatore. The evaluation was carried out with 25 replications, ensuring a robust analysis of the sensory attributes.

Results

Estimation of proximate composition of S. commune Sch.2

The analysis of the nutrient composition of fresh and dried mushrooms revealed that the carbohydrate content (%) was higher in dried mushroom samples compared to fresh samples. Similarly, the protein content in dried mushrooms (13.70%) was more than double that in fresh mushrooms (6.18%). The crude fiber content (%) was also greater in dried mushrooms (3.08%) compared to fresh mushrooms (1.20%). The calorific value (Kcal/100g) was significantly higher in dried mushrooms (358.00) compared to fresh mushrooms (147.84). Total phenols (mg/100g) were found to be highest in dried mushrooms (130.36), followed by fresh mushrooms (108.73). However, the total antioxidant activity was greater in fresh mushrooms (1267.00 µg/g) than in dried mushrooms (1090.76 µg/g). Water activity (aw) was recorded at 0.993 in fresh mushrooms and 0.531 in dried mushrooms.

The mineral composition of the fresh and dried mushroom samples showed that potassium content was 3306.32 ppm and 7593.95 ppm in fresh and dried mushrooms, respectively. Similarly, sodium content was recorded as 571.75 ppm in fresh mushrooms and 677.23 ppm in dried mushrooms, while zinc content was 20.34 ppm in fresh mushrooms and 38.56 ppm in dried mushrooms. The color values of both fresh and dried mushrooms, measured with reference to L a b dE values, indicated that the lightness (L) ranged from 95.39 in fresh mushrooms to 84.46 in dried mushrooms. The redness/greenness (a) values were -1.97 for fresh mushrooms and -2.99 for dried mushrooms, while the yellowness/blueness (b) values were recorded as 25.87 for fresh mushrooms and 17.05 for dried mushrooms (Table 1).

Table 1. Nutritional parameters potential isolate of *S. commune* (*Sch.*2) fruiting bodies

S. No	Parameters		*S. commune*	
			Fresh mushroom	**Dried mushroom**
1	Carbohydrate (%)		30.51	74.00
2	Protein (%)		6.18	13.70
3	Crude fiber (%)		1.20	3.08
4	Calorific value (Kcal/100g)		147.84	358.00
5	Total phenols (mg/100g)		108.73	130.36
6	Total antioxidant activity (µg/g)		1267.00	1090.76
7	Water activity (a_w)		0.993	0.531
8	Potassium (ppm)		3306.32	7593.95
9	Sodium (ppm)		571.75	677.23
10	Zinc (ppm)		20.34	38.56
	Color value	Reference		
11	L	107.73	95.39	84.46
	a	-1.22	-1.97	-2.99
	b	6.24	25.87	17.05
	dE	-	25.60	25.80

Antioxidant assays

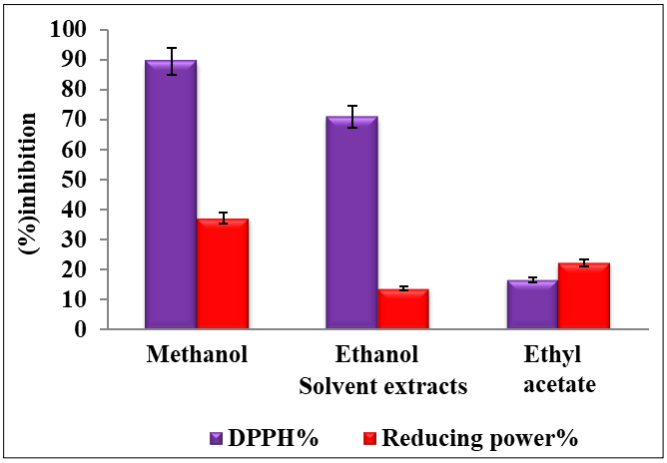
The investigation of the antioxidant activity of different solvent extracts of *S. commune* dried powder at a concentration of 100 µg/mL revealed that the highest DPPH% inhibition was observed in the methanolic extract (89.60%), followed by the ethanolic extract (71.00%), while the ethyl acetate extract exhibited the lowest inhibition activity. An additional assessment of antioxidant potential, conducted using the reducing power assay, demonstrated that the methanolic extract exhibited the highest reducing power ability (37.20%), followed by the ethyl acetate extract (22.30%) and the ethanolic extract (13.80%).

The determination of total phenolic content across the different solvent extracts showed that the methanolic extract had the highest phenolic content (0.230 ± 0.005 mg/mL), followed by the ethanolic extract (0.205 ± 0.005 mg/mL) and the ethyl acetate extract (0.100 ± 0.000 mg/mL) (Fig. 1a).

Phytochemical analysis

The phytochemical analysis, including total flavonoid content (TFC), total tannin content and total saponin content, was conducted for different solvent extracts of *S. commune* dried mushroom and the results are summarized below. The estimation of total flavonoid content across the solvent extracts revealed that the methanolic extract exhibited the highest TFC (0.455 ± 0.003 mg/mL), followed by the ethanolic extract (0.370 ± 0.01 mg/mL) and the ethyl acetate extract (0.252 ± 0.004 mg/mL).

The total tannin content analysis showed that the ethanolic extract recorded the highest tannin content (0.250 ± 0.001 mg/mL), followed by the ethyl acetate extract (0.216 ± 0.001 mg/mL) and the methanolic extract (0.188 ± 0.003 mg/mL). Regarding total saponin content, the ethanolic extract exhibited the highest value (0.303 ± 0.001 mg/mL), followed by the ethyl



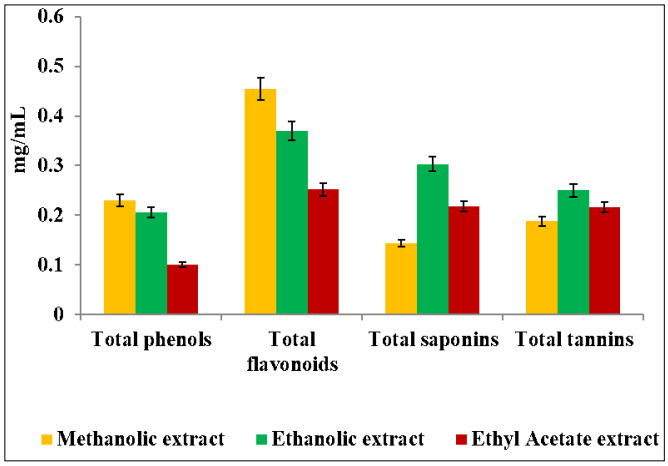


Fig. 1. Antioxidant activity (a) and phytochemical analysis (b) of *S. commune* (*Sch*.2).

acetate extract (0.218 ± 0.001 mg/mL) (Fig. 1b).

Anti-diabetic assay

The glycemic potential of *S. commune* dried mushroom powder was assessed at hourly intervals, revealing a total starch digestibility (TSD) of 7.65% and a glycemic index (GI) of 10.33. These results indicate that *S. commune* mushroom possesses a low glycemic index, making it suitable for consumption as a functional and fortified food. Consequently, its consumption may help prevent a rapid postprandial rise in blood glucose levels.

Development of fortified herbal infusion of S. commune and analysis of antioxidants and phytochemicals

The *S. commune* dried mushroom powder was developed as an herbal infusion using additional herbal ingredients such as dry basil leaf powder and dry ginger powder to enhance its immunomodulatory properties. Three formulations were prepared for evaluation: T1 (*S. commune* dried mushroom powder alone), T2 (*S. commune* dried mushroom powder + dry basil leaf powder + dry ginger powder) and T3 (dry basil leaf powder + dry ginger powder). These formulations were packaged in filter bags, steeped in 100 mL of hot water at 70˚C and subjected to sensory evaluation by 25 participants. The sensory scores for the formulations ranged from "neither liked nor disliked" to "liked very much." The appearance, color, flavor, consistency, taste and overall acceptability of the herbal infusions followed the order T2 > T3 > T1. The highest overall acceptability was observed for T3, with a score of 8.40, followed by T2 (7.30) and T1 (5.80) (Table 2).

Although T3 demonstrated considerable antioxidant and phytochemical properties, the addition of *S. commune* dried powder in T2 further enhanced its nutraceutical properties. Therefore, T2 has the potential to be promoted as a superior product due to its higher antioxidant activity and phytochemical content compared to the other treatments.

To determine the antioxidant activity and phytochemical properties, the herbal infusions were analyzed for DPPH inhibition percentage, total phenolic content, total flavonoid content and total tannin content. The results revealed that the highest DPPH inhibition percentage was observed in T2 (*S. commune* + dry basil leaf powder + dry ginger powder) at 68.50%, followed by T3 (dry basil leaf powder + dry ginger powder) at 44.39% and T1 (*S. commune* alone) at 34.14%. The total phenolic content was also highest in T2 (0.250 ± 0.005 mg/mL), followed by T3 (0.225 ± 0.0005 mg/mL) and T1 (0.200 ± 0.01 mg/mL). Similarly, the total flavonoid content and total tannin content were highest in T2 (0.11 ± 0.038 mg/mL and 0.300 ± 0.01 mg/mL, respectively), followed by T3 (0.10 ± 0.348 mg/mL and 0.280 ± 0.009 mg/mL, respectively) (Fig. 2).

Discussion

Mushrooms are generally nutrient-rich and low in fat, as observed in our study on *S. commune* fresh and dried mushrooms. The proximate composition was recorded as follows: carbohydrate content (74.00% in fresh and 30.51% in the dried mushrooms), protein content (6.18% in fresh and 13.70% in dried mushroom); crude fiber content (3.08 in fresh and 1.20% in dried mushroom) and mineral composition, including potassium (7593.95ppm in fresh and 3306.32 ppm in dried mushroom), sodium (677.23 ppm in fresh and 571.75 ppm in dried mushroom) and zinc (38.56 ppm in fresh and 20.34 ppm in dried mushroom). Similarly, the proximate composition of *S. commune* as ash (7.4 percent), fat (1.2 percent), crude protein (9.6 percent), crude fiber (0.044 percent), carbohydrate (81.5

Table 2. Sensory evaluation of the herbal infusion of *S. commune* (*Sch*.2)

Treatments	Parameters					
	Appearance	Colour	Flavour	Consistency	Taste	Overall acceptability
T1	5.99^{c}	5.66^{c}	5.33^{c}	5.99^{c}	5.99^{c}	5.80^{a}
T2	7.30^{b}	7.33^{b}	7.50^{b}	7.99^{b}	7.80^{b}	7.30^{b}
T3	8.00^{a}	8.50^{a}	8.60^{a}	8.50^{a}	8.30^{a}	8.40^{c}
SEd	0.12	0.05	0.07	0.06	0.07	0.06
CD (0.05)	0.26	0.11	0.15	0.12	0.18	0.13

Values are means of twenty-five replications. The means followed by the same letter are not significantly different from each other by DMRT (P=0.05). T1: *S. commune* dried mushroom powder alone, T2: *S. commune* dried mushroom powder + dried basil leaf powder + dried ginger powder and T3= Dried basil leaf powder + dried ginger powder.

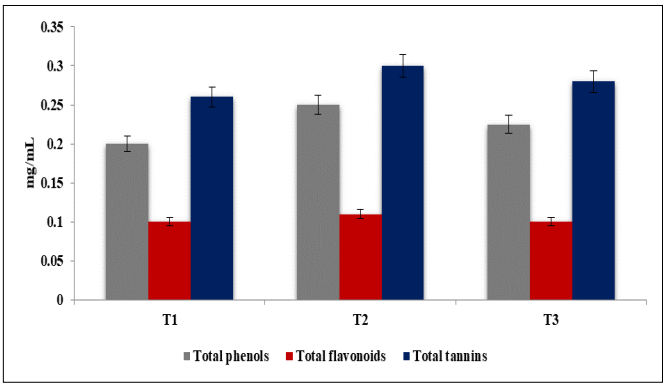


Fig. 2. Phytochemical analysis of *S. commune* (*Sch*.2) fortified herbal infusion. T_1= *S. commune* dried mushroom powder alone, T_2= *S. commune* dried mushroom + dried basil leaf powder + dried ginger powder and T_3= Dried basil leaf powder + dried ginger powder.

percent). They also reported the vitamin content (mg/100g dry wt basis) as ascorbic acid (0.49), niacin (1.30), riboflavin (0.22), thiamine (0.2 (0.375) (25). *S. commune* exhibited the highest vitamin A content (2711.30mg/g) and vitamin E content (85.08mg/g) (26). The proximate composition of *S. commune*, including fat (2.28%), protein (7.69%), fiber (10.84%), ash (2.44%) and carbohydrate (75.70 %) which is similar to our study (27).

These studies indicate that *S. commune* is low in fat, rich in fiber, protein and vitamins and can serve as a functional food ingredient. A comparative study on *Lentinus edodes* showed its proximate composition as protein (22.8 g/100 g), fat (2.1 g/100 g), carbohydrate and fiber (64.4 g/100 g), energy (411 kcal) and minerals such as phosphorus (439 mg), zinc (4.3 mg), magnesium (200 mg) and calcium (127 mg) (28). Similarly, the study revealed that the proximate composition of *Pleurotus* mushrooms cultivated on paddy straw substrate included protein (23.40%), fat (2.8%), carbohydrate (55.33%), fiber (7.7%) and minerals such as phosphorus (920 mg), sodium (290 mg) and calcium (296 mg) (29). These findings highlight that *S. commune* possesses a proximate composition comparable to other nutritionally significant mushrooms, reinforcing its potential as a functional fortified food.

The evaluation of solvent extracts (methanol, ethanol and ethyl acetate) revealed that all solvents successfully extracted nutraceuticals, but the methanolic extract demonstrated the highest DPPH inhibition activity (89.60%), followed by the ethanolic extract (71.00%).

A similar study reported a 50% DPPH scavenging activity in ethanolic extracts of *S. commune* (14). Conversely, high reducing power ability was observed in hot water extracts of *S. commune*, followed by ethanolic extracts (4). However, our study found that the methanolic extract exhibited the highest reducing power ability (37.20%), followed by ethyl acetate (22.30%) and ethanol (13.80%). Similarly, the total phenolic content was highest in the methanolic extract (0.230 mg/mL). Higher phenolic content (9.49 mg GAE/g extract) were reported in ethanolic extracts of *S. commune* (30). In our analysis, the total phenolic content (0.230±0.005 mg/mL) and total flavonoid content (0.252±0.004 mg/mL) were highest in the methanolic extract, while total tannin content (0.25±0.001 mg/mL) and total saponin content (0.303±0.001 mg/mL) were highest in the ethanolic extract.

The supporting study analysed that the antioxidant activity and total phenolic content of *Volvariella volvacea* and *S. commune*. Radical scavenging activity was recorded at 21.19% in *V. volvacea* mycelia grown in coconut water and 19.45% in *S. commune* cultured in rice bran broth. The highest phenolic content was observed in rice bran broth substrate for *V. volvacea* mycelia (23.19 mg AAE/g) and in coconut water for *S. commune* (25.52 mg GAE/g sample) (31). The study identified secondary metabolites in *S. commune*, including flavonoids, steroids, tannins and coumarins, with an antioxidant activity of 121.37 g/mL (32). The study reported that acetone extracts of *Pleurotus ostreatus* exhibited higher total phenolic content and antioxidant activity, along with moderate levels of terpenoids, tannins and steroidal glycosides (33).

In addition to antioxidant and phytochemical analysis, the glycemic potential of *S. commune* was assayed, revealing a total starch digestibility of 7.5% and a glycemic index of 10.33. Generally, the glycemic index is a measure of how carbohydrate-containing foods affects blood sugar levels; a low glycemic index indicates slower glucose release, reducing the risk of postprandial blood sugar spikes. A comparable study on *L. edodes* reported slow digestible starch (18.23%) (34).

A study on methanolic extracts of *S. commune* exhibited the highest antidiabetic activity, with IC50 = 50.98 µg/mL. The low GI of *S. commune* suggests its suitability as a functional food, particularly for diabetic patients (10).

Sensory evaluation results revealed that treatment T3 (dry basil leaf powder + dry ginger powder) had the highest overall acceptability, followed by T2 (*S. commune* + dry basil leaf powder + dry ginger powder) and T1 (*S. commune* alone). The antioxidant potential of T2 was significantly enhanced due to the inclusion of *S. commune*. Although T3 achieved high acceptability due to familiarity with its taste, T2 demonstrated enhanced nutraceutical properties with increased antioxidant activity. T1 was also accepted by 25% of participants due to its consistent mushroom flavor, achieving an overall acceptability score of 5.80.

A study on fruit tea mixed with *S. commune* reported that a formulation containing pineapple, salak and longan (20:20:60) with 20% *S. commune* powder achieved high consumer acceptance, with a sensory score of 7.46 (35). Similarly, the present study suggests that T1 and T2 formulations can be promoted as fortified products to deliver nutraceutical benefits to consumers. The analysis of antioxidant activity and phytochemical content revealed that T2 exhibited the highest DPPH inhibition percentage (68.50%), total phenolic content, total flavonoid content and total tannin content, followed by T3 and T1. These results further support the potential of *S. commune* as a functional food ingredient for product development.

Conclusion

The conclusion of the current study indicates that *S. commune* contains significant levels of carbohydrates, crude fiber and crude protein, along with notable antioxidant properties, making it considered edible, safe and suitable for consumption. The nutraceutical properties of *S. commune*, when extracted with various solvents, demonstrated the highest DPPH inhibition percentage, along with increased flavonoid and saponin content. The primary objective of the study was to develop a suitable herbal infusion using *S. commune*. The combination of dried

S. commune powder, dried basil leaf powder and dried ginger powder exhibited high antioxidant and phytochemical properties. Sensory evaluation of the herbal infusion revealed a preference for its bitter taste.

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Authors' Contributions

RM carried out the isolation, characterization, cultivation of *S. commune* and drafted the manuscript. TG investigated proximate composition and nutraceutical analysis of *S. commune.* AK, AD and GP performed the sensory evaluation of the herbal extract. HKS participated in the evaluation of antioxidant activities. PT drafted the manuscript, review, editing and performed statistical analysis. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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