Metabolite Profiling of Ethanolic Extracts from Dried Cremini Mushrooms: A Qualitative and Quantitative Study

Ch. Kethani Devi and P. Sudhakar✉

Mobile Number: +91 9493101093

Department of Biotechnology, Acharya Nagarjuna University, Guntur–522 510, Andhra Pradesh, India.

Abstract

The ethanolic extract of dried cremini mushrooms (*Agaricus bisporus* var. *cremini*) was analyzed for its phytochemical composition using qualitative and quantitative methods. Qualitative screening was performed to identify major bioactive compounds, including flavonoids, alkaloids,, tannins, terpenoids, phenols, saponins, glycosides, and steroids, using standard phytochemical tests. Quantitative estimation was carried out to determine the concentrations of total phenolics (Folin-Ciocalteu method), flavonoids (aluminum chloride method), and other significant phytoconstituents via spectrophotometric analysis. The results revealed the presence of diverse phytochemicals, with notable levels of flavonoids and phenolic compounds, which are known for their antioxidant potential. This study highlights the therapeutic value of cremini mushrooms and supports their use in nutraceutical and pharmacological applications. Primary metabolites total carbohydrates and totals proteins were estimated by anthrone and biuret methods respectively.

Keywords: *Agaricus bisporus*, phytochemical screening, total phenolic content, total flavonoids, ethanolic extract, cremini mushrooms.

INTRODUCTION

Brown edible mushrooms are a popular variety of fungi known for their rich flavor, firm texture, and numerous culinary uses. These mushrooms, which include types like cremini, portobello, and shiitake, are commonly found in kitchens around the world and are prized for their earthy taste and nutritional benefits. Cremini mushrooms, also known as baby Bellas, are younger versions of the larger and more mature portobello mushrooms.

According to Heinemann (1978), the Cremini and Portobello mushroom are classified within the Fungi kingdom. Their complete taxonomic hierarchy is as follows: they belong to the phylum Basidiomycota, the class Agaricomycetes, the order Agaricales, and the family Agaricaceae. These common edible mushrooms are classified under the genus Agaricus and are both the species Agaricus bisporus.

They belong to the same species as the common white button mushroom (*Agaricus bisporus*) but are harvested at a slightly later stage, giving them a darker color and deeper flavor. Shiitake mushrooms, on the other hand, are native to East Asia and are distinguished by their umbrella-shaped brown caps and rich umami taste. Nutritionally, cremini mushrooms are high in important nutrients such as selenium, B vitamins, and antioxidants but low in calories. They provide a good source of dietary fiber and connected to numerous health benefits, including immune support and anti-inflammatory properties.

In cooking, brown mushrooms are versatile and can be sautéed, grilled, roasted, added to soups, sauces, and stir-fries. Their robust flavor makes them an excellent meat substitute in vegetarian dishes [2], brown edible mushrooms are a valuable addition to a healthy diet and a flavorful ingredient in diverse cuisines. Cremini mushrooms (*Agaricus bisporus*) possess a rich biochemical [3] and bioactive composition that contributes to both their nutritional value and potential health benefits. Biochemically, they are composed of about 90 % water, with low levels of fats and moderate amounts of carbohydrates and proteins. Bioactive components [4] such as **β-glucans** and **chitin**, which support immune function and gut health. Among the most notable bioactive compounds are **ergothioneine**, a powerful antioxidant amino acid derivative, and **phenolic compounds**, which contribute to their anti-inflammatory and

antioxidant properties. Other functional molecules like **lectins** and **terpenoids** also exhibit potential anticancer and immunomodulatory effects, making cremini mushrooms a valuable functional food.

Carbohydrates in cremini mushrooms are primarily structural, forming the cell walls and contributing to the mushroom's overall structure. They also play a role in the mushroom's growth and fruiting process, being converted and used as an energy source. Some of the glucose from the mycelium is converted into trehalose and mannitol, which act as energy sources during reproduction and development. Additionally, certain carbohydrates, like glucans, have beneficial biological effects, including potential anti-cancer and anti-inflammatory properties. While not a primary source of energy for humans, the carbohydrates in cremini mushrooms contribute to their nutritional profile and overall health benefits.

Cremini mushrooms containing essential amino acids crucial for various bodily functions and provide a good source of plant-based protein. They contain all the essential amino acids, particularly high in branched-chain ones like leucine, which can help reduce muscle fatigue and pain. Beyond their nutritional value, mushroom proteins also boast bioactive peptides with potential health benefits, contributing to their role as a viable alternative to animal protein sources.

Phytochemical composition

Phytochemicals, derived from the Greek word *phyton* (plant), are naturally occurring plant substances with beneficial or detrimental health effects, and medicinal plants are the richest bio-reservoirs of diverse phytochemicals used to treat various illnesses, with therapeutic qualities determined by components like alkaloids, flavonoids, tannins, saponins, steroids, and terpenes found in different plant regions. Cremini mushrooms produce phytochemicals through primary and secondary metabolism; these substances benefit plants by shielding them from stress, pollution, drought, and diseases, while also aiding blossoming and repelling pests, with primary metabolites (proteins, amino acids, sugars) overseeing fundamental growth and secondary metabolites (flavonoids, saponins, alkaloids) providing flavor, color, aroma, and enabling survival in hostile environments, utilized commercially for coloring, insecticides, and medications, and protecting humans against aging, diabetes, cancer, and other illnesses [5].

Primary metabolites: Are substances such as carbohydrates, proteins, lipids, and nucleic acids necessary for an organism's growth, development, and reproduction, while secondary metabolites serve ecological purposes like defense against viruses or herbivores, attracting pollinators, or influencing species relationships, though not directly necessary for existence [6].

Proteins: Are vast molecules made of amino acids, containing carbon, hydrogen, oxygen, and nitrogen, and are essential for metabolic activities, stimulus response, and cell structure [6].

Amino acids:, Are chemically organic molecules containing both a basic amine group (-NH2) and an acidic carboxyl group (-COOH) and a specific side chain (R group), composed of nitrogen (N), oxygen (O), hydrogen (H), and central carbon (C), and are crucial for functions like biosynthesis and nerve cell communication [7].

Aromatic amino acids: Like tryptophan and tyrosine are detected via the xanthoproteic reaction, where their aromatic rings react with concentrated nitric acid to form yellow xanthoproteic acid, turning orange with alkali, indicating their presence; this test is used for protein identification and quality control in food, pharmaceuticals, and clinical research [8].

Carbohydrates: Produced by plants through photosynthesis, are composed of carbon (C), hydrogen (H), and oxygen (O) and serve as energy sources in foods like bread, fruits, and vegetables, containing starch and cellulose [9]. Reducing sugars, such as fructose, glucose, maltose and lactose, have free ketone or aldehyde groups that act as reducing agents, vital for energy production and processes like the Maillard reaction [10].

Starch: Is a polysaccharide of glucose units, serving as the primary energy storage in plants, found in seeds, roots, and tubers, and consists of amylose and amylopectin that determine its structure and digestibility [11].

Cholesterol: Is a lipid sterol crucial for hormone production, vitamin D synthesis and cell membrane structure, synthesized in the liver and acquired from dietary sources like dairy and meat, with elevated levels linked to cardiovascular diseases [12].

Secondary metabolites: Are organic molecules produced by plants and microorganisms, crucial for ecological interactions like defense against herbivores, pathogens, and predators, but not essential for direct growth, development, or reproduction; examples include terpenoids, alkaloids, and phenolics, which are species-specific and often generated in small amounts during stationary development phases.

Tannins: Are polyphenolic compounds in cremini mushrooms that act as natural antioxidants and antimicrobial agents, defending against microbial infections and environmental stress while offering human health benefits like antimicrobial properties, antioxidant [13], anti-inflammatory [14] enhancing nutritional and medicinal value.

Flavonoids: In cremini mushrooms provide anti-inflammatory and antioxidant benefits, combating supporting immune function, reducing inflammation, oxidative stress and potentially lowering chronic disease risk [15].
Terpenoids: Aid defense by repelling pests and inhibiting microbes, contributing to aroma and biological activity, with human health benefits including antioxidant, anti-inflammatory, and potential anticancer properties [16].
Saponins: Exhibit antifungal and antibacterial properties, protecting against pathogens and contributing to bitterness and foaming, while offering human benefits like cholesterol lowering, immune boosting, and anti-inflammatory and anticancer activity [17].
Steroids: Are lipids with a four-ring structure, including hormones like testosterone and cortisol that regulate metabolism, reproduction, and immune responses, with synthetic forms used as anti-inflammatory agents and anabolic steroids [18].
Phlobotanins: classified by structure and function, include hydrolysable tannins (gallic or ellagic acid bound to sugars) and condensed tannins (flavan-3-ol polymers), essential for plant defense, UV protection, and nutrient cycling, with human antibacterial, anti-inflammatory, and antioxidant properties, and improved extraction methods like deep eutectic solvents [19].
Alkaloids: Are nitrogen-containing compounds in cremini mushrooms that defend against pests and pathogens, with neuroactive properties potentially supporting cognitive health and mood regulation, along with antimicrobial and anticancer effects [20].
Emodin: Is an anthraquinone ($C_{15}H_{10}O_5$) found in plants like rhubarb with antibacterial, anticancer properties, anti-inflammatory, antioxidant and benefits for cardiovascular disorders by targeting oxidative stress and inflammation [21].
Anthraquinones: Feature a 9,10-anthraquinone scaffold and are found in plants, fungi, and lichens, with derivatives like emodin and aloe-emodin offering broad biological and pharmacological properties [22].
Anthocyanins: Are water-soluble flavonoids providing red, purple, and blue hues in plants, with oxidative stress-reducing antioxidant properties, inflammation, and prevent neurological and cardiovascular disorders [23].
Coumarins: Are aromatic compounds with a benzopyrone structure, found in spices and fruits offering anticoagulant, , antimicrobial, anti-inflammatory and antioxidant properties, and serving as drug precursors like warfarin, though high levels can be toxic [24].
Phenolic components: In cremini mushrooms protect against oxidative damage and pathogens, acting as antioxidants with human anti-inflammatory, antimicrobial, and anticancer benefits, enhancing nutritional value [25].
Glycosides: Consist of a sugar linked to an aglycone via a glycosidic bond, functioning as signaling molecules, antioxidants, and antimicrobials in biological systems, with medicinal uses like cardiac glycosides for heart disease and potential anticancer applications [26].

Materials and Methods

Collection of plant materials: *Agaricus bisporus,* commonly known as the brown button mushrooms, was sourced from the Lambasingi area in Visakhapatnam. The cremini mushrooms were dried 72 hours under shaded conditions to preserve their bioactive compounds, finally using a high-speed blender ground into a fine powder.

Sample preparation: After dissolving 10 grams of the dry powder in 100 mL of ethanol, the mixture was put in an orbital shaker set to 40 degrees Celsius and 3000 rpm for 48 hours. Then using a Buchner funnel the solution was filtered to remove any solid residue. Using a rotary evaporator under reduced pressure, the ethanol was then evaporated, concentrating the extract. The crude extract was purified and characterized for the analysis of primary and secondary metabolites.
After ethanolic extraction, the residue was collected from buchner funnel and mixed with hot water and contuned heating for 2 hours at 60 ^{0}C in a round bottom flask. Acquous extract and solid residue was seperated from the mixture using vaccum filtration. The extract was concentrated by evaporating the water under reduced pressure using a rotary evaporator. This concentrated aqueous solution was then used to quantify the total proteins in the cremini mushrooms.

Table : 1. Qualitative phytochemical screening

Qualitative test		Procedure	Observation
Primary Metabolites	Protein (Biuret test)	1 mL NaOH + 2 mL extract → wait 2 min → add 1 mL $CuSO_4$.	Violet or purple color
	Amino Acids	1 mL extract + 2 mL ninhydrin → boil for 10 min.	Blue or purple color
	Aromatic amino acids (Xanthoproteic test)	1 mL extract + 5 drops HNO_3 → heat 3 min → add NaOH.	Orange or yellow color
	Carbohydrates (Molisch's test)	1 mL extract + 3 drops α-naphthol → mix → add H_2SO_4 along sides.	Purple ring at interface
	Reducing sugars	2 mL extract + 3 mL Benedict's → heat 5 min in water bath.	Brick to red precipitate
	Starch	1 mL extract + 6 drops iodine.	Blue to black color
Secondary Metabolites	Cholesterol	1 mL extract + 1 mL $CHCl_3$ + 1 mL acetic anhydride → add H_2SO_4.	Green or blue color
	Tannins	Few drops $FeCl_3$ + 2 mL extract + 2 mL H_2O.	Dark blue or green or black precipitate
	Flavonoids	2 mL extract + 3-5 drops NaOH → dilute with HCl.	Yellow to colorless (on acidification)
	Terpenoids	2 mL extract + 2 mL acetic anhydride → add 2-3 drops H_2SO_4.	Reddish brown ring
	Saponins	1 mL extract + 1 mL lead acetate.	White precipitate
	Steroids	2 mL extract + 2 mL chloroform → add H_2SO_4 sidewise.	Red upper layer, green or yellow fluorescence
	Phlobotanins	1 mL extract + 2 mL HCl → heat.	Red precipitate
	Alkaloids	1 mL extract + 1 mL Hager's reagent.	Yellow precipitate
	Emodin	2 mL extract + 2 mL NH_4OH + 3 mL benzene.	Pink/red color in benzene layer
	Anthraquinones	3 mL extract + 3 mL benzene → add 5 mL ammonia.	Pink/red color in ammonia layer
	Anthrocyanins	2 mL extract + 2 mL HCl → heat → add 2 mL ammonia.	Blue or green color
	Coumarins	2 mL extract + 3 mL NaOH.	Yellow fluorescence
	Phenolic components	2 mL extract + 2 mL $FeCl_3$.	Blue or green or black color
	Glycosides	2 mL extract + 2 mL $CHCl_3$ + 2 mL acetic acid → ice for 5 min → add H_2SO_4 slowly.	Reddish brown ring
	Phenol	Few drops lead acetate + extract.	White precipitate

Quantitative estimation of Secondary metabolites

1. Quantitative estimation of total Alkaloids: The total alkaloid content in the ethanolic extract of cremini mushrooms was quantitatively estimated using the bromocresol green (BCG) complexation method. Standard solutions (20–100 µg/mL) were used to construct a calibration curve. For analysis, 1 mL of the extract was mixed with 5 mL of phosphate buffer (pH 4.7) and 5 mL of BCG solution to form an ion-pair complex, which was extracted with 4 mL of chloroform. The yellow chloroform layer was collected, diluted to 10 mL, and its absorbance measured at 470 nm. The total alkaloid concentration, expressed as atropine equivalents, was determined by interpolating the absorbance value into the linear regression equation of the standard curve.

$$\text{Alkoloids (mg/100 g)} = \frac{\text{Alkoloids (µg/mL) x Dilution factor x Volume sample (mL)}}{\text{Weight of sample taken}}$$

2. Quantitative estimation of total phenolic components (TPC) : The total phenolic content (TPC) of the ethanolic extract of cremini mushrooms was determined using the Folin-Ciocalteu method, with gallic acid as the standard. A calibration curve was prepared using gallic acid solutions (20–100 µg/mL). For the assay, 1 mL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent, followed by the addition of 10 mL of 7% sodium carbonate and 13 mL of distilled water after 5 minutes. The mixture was incubated in the dark for 90 minutes to allow color development, and absorbance was measured at 765 nm. The total phenolic content, expressed as gallic acid equivalents (GAE µg/mL), was determined by interpolating the absorbance into the linear regression equation of the gallic acid standard curve.

3. Quantitative estimation of Flavonoids: The total flavonoid content (TFC) of the ethanolic extract of cremini mushrooms was determined using the aluminum chloride colorimetric method, with quercetin as the standard. A calibration curve was prepared using quercetin solutions (20–100 µg/mL). For the assay, 1 mL of the extract was mixed with 4 mL of distilled water, followed by the sequential addition of 0.3 mL of 5% sodium nitrite, 0.3 mL of 10% aluminum chloride (incubated for 6 minutes), and 2 mL of 1 M sodium hydroxide. The final volume was adjusted to 10 mL with distilled water to form a flavonoid–aluminum complex. Absorbance was measured at 510 nm, and the total flavonoid content, expressed as quercetin equivalents (µg QE/mL), was calculated by interpolating the sample absorbance into the linear regression equation of the quercetin standard curve.

4. Quantitative estimation of Tannin: The total tannin content (TTC) of the ethanolic extract of cremini mushrooms was determined using the Folin-Ciocalteu colorimetric method, with tannic acid as the standard. A calibration curve was prepared using tannic acid solutions (20–100 µg/mL). For the assay, 1 mL of the extract was mixed with 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 35% sodium carbonate in a 10 mL volumetric flask. The mixture was kept at room temperature for 30 minutes to allow color development, and absorbance was measured at 700 nm. The total tannin content, expressed as tannic acid equivalents (µg TAE/mL), was calculated by interpolating the absorbance value into the linear regression equation of the standard curve.

5 Quantitative estimation of Terpenoids: The total terpenoid content (TTC) of the ethanolic extract of cremini mushrooms was determined using the anisaldehyde–sulfuric acid colorimetric method. A calibration curve was prepared using terpenoid standard solutions (20–100 µg/mL). For the assay, 1 mL of the extract was mixed with 2.5 mL of anisaldehyde reagent, incubated at 60 °C for 10–15 minutes, and then cooled to room temperature. The absorbance of the resulting-colored complex was measured at 540 nm. The terpenoid content, expressed as terpenoid equivalents (µg TE/mL), was calculated by interpolating the sample absorbance into the linear regression equation of the standard curve.

Quantitative estimation of primary metabolites

Quantitative estimation of total carbohydrates by Anthrone method: The total carbohydrate content of the plant extract was determined using the anthrone–sulfuric acid method, with D-glucose as the standard. A glucose stock solution (2000 µg/mL) and anthrone reagent (0.200 mg in 100 mL concentrated sulfuric acid) were prepared. Standard solutions containing 20–100 µg of glucose were diluted to 1 mL with distilled water, mixed with 4 mL of ice-cold anthrone reagent, and heated in a boiling water bath for 10 minutes to form a blue-green complex. Absorbance was measured at 620 nm to construct a calibration curve. The sample was processed similarly, and its carbohydrate content, expressed as glucose equivalents (µg GE), was determined by interpolating its absorbance into the linear regression equation of the standard curve.

Quantitative estimation of total proteins by biuret method : The total protein content of the extract was determined using the Biuret method, with bovine serum albumin (BSA) as the standard. The Biuret reagent was prepared by dissolving 3 g of $CuSO_4$, 9 g of sodium potassium tartrate, and 5 g of KI in 0.2 N NaOH and making up the volume to 1000 mL. Standard solutions were prepared from a 5000 µg/mL BSA stock by pipetting 0.4–1.6 mL aliquots, adding

3 mL of Biuret reagent, and incubating at 37°C for 10 minutes to form a violet-colored complex. Absorbance was measured at 540 nm. The sample was treated similarly, and its protein concentration, expressed as BSA equivalents (µg BSAE), was calculated by interpolating the absorbance into the linear regression equation of the BSA standard curve.

RESULTS AND DISCUSIION

Table: 2. Qualitative phytochemical screening

Phytochemical component		Cremini mushroom ethanolic extract
Primary Metabolites	Amino acids	+++
	Carbohydrates	+++
	Reducing sugar	++
	Proteins	++
Secondary Metabolites	Alkaloids	+
	Terpenoids	+++
	Flavonoids	+++
	Tannins	+++
	Phytosterol	+
	Phenol	+++
	Phenolic components	+++
	Anthraquinones	-
	Coumarins	-
	Steroids	-
	Saponins	-

Discussion : Table 2 represents the results of the phytochemical screening tests provided valuable insights into potential bioactivity and the composition and of the ethanolic extract of cremini mushrooms. They show the presence of phytochemical constituents like amino acids, carbohydrates, proteins, and sugars, which are essential compounds of plant and fungal extracts play vital roles in various metabolic processes [27]. The presence of these compounds in the extract indicates their nutritional value and potential benefits [28]. Alkaloids, nitrogen-containing compounds, are often associated with pharmacological activities [29]. Terpenoids, flavonoids, tannins, and phenols in the cremini mushroom extract were known for their anti-inflammatory [30-31]antioxidant, antimicrobial properties.

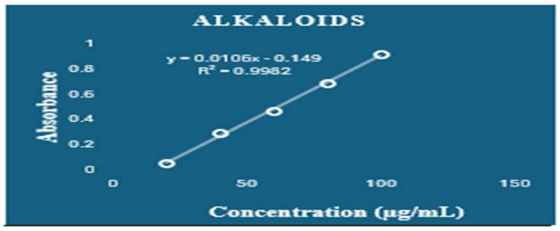


Figure 1(a) Separation Alkaloids Figure 1 (b):Standard Calibration Curve of Alkaloids

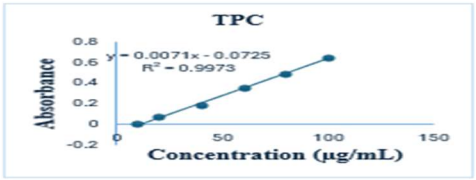


Figure: 2 Standard Calibration Curve of TPC

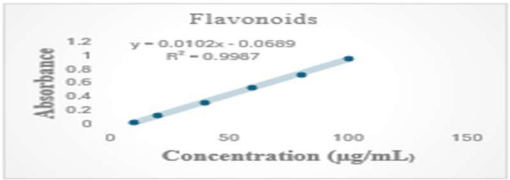


Figure: 3 Standard Calibration Curve of Flavonoids

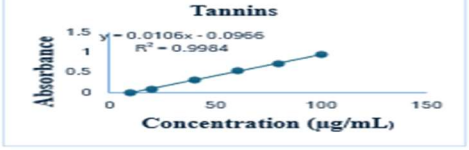


Figure: 4 Standard Calibration Curve of Tannins

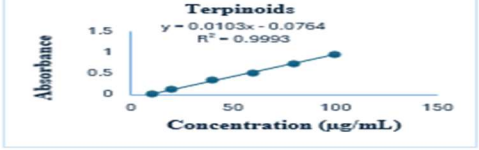
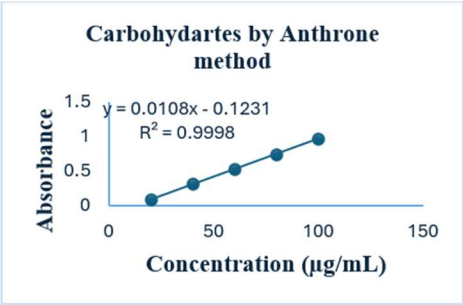


Figure: 5 Standard Calibration Curve of Terpenoids



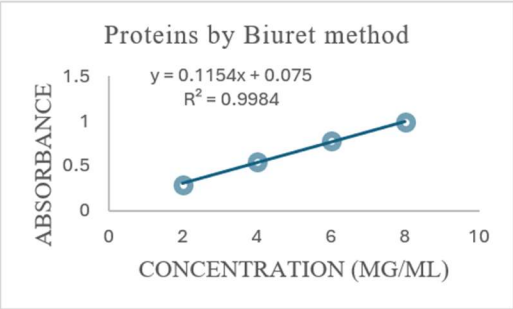


Figure: 6 Standard Calibration Curve of Carbohydrates Figure: 7 Standard Calibration Curve of Proteins

Table : 3. Quantitative phytochemical screening

Name of the phytochemical component		Amount
Primary metabolites	Carbohydrates (g/100g)	1.241
	Proteins (g/100g)	2.02
Secondary metabolites	Alkaloids (mg/g)	1.19
	Total phenolic components (mg GAE /g)	4.79
	Flavonoids (mg QE/g)	4.3
	Tannins (mg/g)	4.35
	Terpenoids (mg/g)	0.481

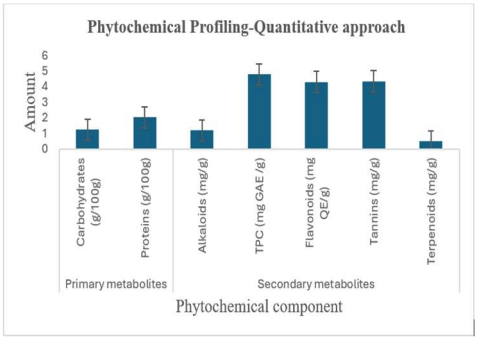


Figure : 8 Phytochemical Profiling-Quantitative approach

Discussion : The quantitative phytochemical screening of cremini mushrooms revealed a notable composition of both primary and secondary metabolites. Among the primary metabolites, proteins (2.02 g/100 g) were present in higher concentration compared to carbohydrates (1.241 g/100 g), which is consistent with the nutritional profile of mushrooms as a low-carbohydrate but protein-rich food source [27]. This highlights their potential role as a healthy dietary option for individuals seeking nutrient-dense, low-calorie foods [28]. In terms of secondary metabolites, flavonoids (4.3 mg QE/g), phenolic compounds (4.79 mg GAE/g), and tannins (4.35 mg/g) were observed in considerable amounts, indicating strong antioxidant potential [30, 31). These bioactive compounds are known to contribute to free radical scavenging, anti-inflammatory effects, and protection against oxidative stress–related diseases [31]. Alkaloids (1.19 mg/g) were present at moderate levels, suggesting potential antimicrobial and therapeutic activities [29], while terpenoids (0.481 mg/g), though lower in concentration, may still contribute to medicinal properties such as anticancer [29]anti-inflammatory activities. Collectively, these findings demonstrate that cremini mushrooms not only serve as a valuable source of essential nutrients but also possess a diverse range of secondary metabolites with promising health-promoting properties.

CONCLUSION: Qualitative and quantitative screening

The study began with a qualitative and quantitative analysis of cremini mushroom (*Agaricus bisporus*) ethanolic extract.

Qualitative Screening: The extract tested positive for a wide range of primary metabolites (amino acids, carbohydrates, reducing sugars, proteins) and secondary metabolites (terpenoids, flavonoids, tannins, phenols, phenolic components). It showed a weak presence of alkaloids and phytosterols and was negative for anthraquinones, coumarins, steroids, and saponins. This profile suggests a high nutritional value and potential for bioactive properties like antioxidant and anti-inflammatory effects.

Quantitative Screening : The comprehensive phytochemical and mineral analysis establish cremini mushrooms as a highly nutritious food source. They are rich in proteins, essential minerals (especially potassium, iron, and zinc), and a wide range of antioxidant bioactive compounds (tannins, phenolics, flavonoids,). The quantification of these components provides a strong scientific basis for promoting their consumption as part of a healthy diet and for their use as a potent natural antioxidant ingredient in the functional food and nutraceutical industries.

APPLICATION

Qualitative screening and quantitative estimation of phytochemicals are essential in establishing correlations between the chemical composition of plant extracts and their biological activities. Preliminary qualitative analysis enables the identification of diverse classes of secondary metabolites, while quantitative determination provides precise measurements of their abundance. These complementary approaches not only validate the pharmacological relevance of detected compounds but also strengthen the interpretation of experimental outcomes by linking metabolite levels to antioxidant, antimicrobial, or other bioactivities. Thus, they serve as a foundation for elucidating mechanisms of action and advancing bioactive compounds toward therapeutic applications.

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