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TAXONOMY, PHYSICOCHEMICAL PROPERTIES AND MYCOCHEMICAL COMPOSITION OF WOOD ROTTING MUSHROOM *PHELLINUS PACHYPHLOEUS* (PAT.) PAT.

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ABSTRACT: The genus *Phellinus* Quél. (Family-*Hymenochaetaceae*) is a cosmopolitan polypore mushroom including a large number of wood rotting species. However some of these species have pharmacological significance and are in use as folk medicine since ages. The present investigation aims at taxonomic study, evaluation of physicochemical properties and screening of mycochemical composition of the specimen collected from district Dehradun, Uttarakhand (India). Based on morphology and microscopy, the specimen was identified as *Phellinus pachyphloeus* (Pat.) Pat. This mushroom showed negligible foreign matter, low moisture content (13.67%), high dry weight (86.33%), good flow characteristics, high dispersibility (85.67%), high alcohol soluble extractives, high water absorption capacity, good emulsion properties and high content of water soluble ash (3%). The preliminary mycochemical screening was done as per established standards and showed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenols, flavonoids, tannins, anthraquinone glycosides and cardiac glycosides. These results for *Phellinus pachyphloeus* (Pat.) Pat. are an indication of its potential use in pharmaceutical and nutraceutical preparations.

KEYWORDS: Mycochemical; *Phellinus pachyphloeus*; Physicochemical; Uttarakhand.

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1. INTRODUCTION

Traditional use of mushrooms as folk medicine and for culinary purposes is well established [1]. Of these mushrooms, species belonging to genus *Phellinus* occupy an important place as therapeutic agents [2]. There are 180 species of this genus worldwide [3]. India has a vast diversity of *Phellinus* represented by 95 species [4]. These cause wood decay of various angiosperms and gymnosperms [5–7] while some species of this genus have pharmacological significance. *Phellinus rimosus* has been used for the treatment of mumps by the people of local tribes in Kerala [8]. *Phellinus durissimus* is a wood rotting mushroom and is used in traditional medicinal practices by the tribes of Dang district of South Gujarat [9]. *Phellinus* species are good sources of carbohydrates, proteins, fibres, fats and minerals [10–13]. These are rich in various bioactive constituents like polysaccahrides, alkaloids, tannins, flavonoids, phenols, terpenoids and anthocyanins [14–15]. These bioactive constituents attribute to their significant biological activities such as anti-allergic [16, 17], anti-cancer [18–22], anti-diabetic [23], anti-inflammatory [24], anti-microbial [25–27], anti-oxidant [28–29], hepatoprotective [30] and immuno-stimulation [31– 32]. However physicochemical and biochemical characterization have not been evaluated for all the species belonging to *Phellinus*. In the present investigation taxonomy, standardization of physicochemical properties and screening of mycochemical composition was performed on wild specimen of *Phellinus pachyphloeus* (Pat.) Pat.

2. MATERIAL AND METHODS

2.1. Collection of specimen

The collection was done from district Dehradun, Uttarakhand (India) in the month of September during 2012. *Phellinus* species occur in parasitic or saprophytic association with a wide range of angiosperm and gymnosperm hosts causing mainly white rot. In field collections belonging to this genus could be recognized by brown colored basidiome, woody hard texture and positive xanthochroic reaction (pore surface turning black in KOH solution). The name of locality, name and type of host, date of collection and type and attachment of basidiome was noticed. Field photography of the specimen was also done. The collection was dried in shade or with an electric drier at 40°C–45°C it was then packed in cellophane packets/zip lock polythene bags after putting 1, 3-Dichlorobenzene crystals to prevent microbial attack.

2.2. Morphology of the specimen

Morphological details of basidiome pertaining to its upper and lower surface, margins, consistency, thickness, type and color of context, pore tubes and number of pores were observed with hand lens. The color standards used were in accordance with the Methuen's Handbook of colors [33]. Spore prints of fresh specimen were taken for the detailed study of spores.

2.3. Microscopy of the specimen

Microscopy pertaining to the type and dimensions of hyphae, basidia, basidiospores, setae and setal hypahe was done by making crush mounts and free hand sections in water as well as 3%/5%/10%

potassium hydroxide solutions followed by their staining in 1% Phloxine and 1% Congo red. The amyloid/dextrinoid and cyanophilous reactions of spores were noticed with Melzer's reagent (0.5g Iodine, 1.5g Potassium iodide, 20g chloral hydrate and 20 mL distilled water) and cotton blue (1% in lactophenol) respectively. Microscopic structures were observed using compound light microscope at different magnifications 10x × 10x, 10x × 40x, 10x × 100x (oil immersion lens) and line diagrams of these structures were made on white sheets using camera lucida [34].

2.4. Documentation

The observations regarding morphology and microscopy of the specimen were compiled in the form of a description. This description was then compared with the published literature and the specimen was physically compared with the type material lying at the Herbarium, Botany Department, Punjabi University, Patiala and Herbarium, Forest Research Institute, Dehradun for identification.

2.5. Submission of specimen

The specimen after identification was assigned a unique herbarium number (PUN 5995) and was deposited at the internationally recognized herbarium of Department of Botany, Punjabi University, Patiala.

2.6. Glassware and chemicals

The Corning/Borosil glassware were used during experimentation. All chemicals used were of AR grade and were purchased from Himedia, Loba chemie and SD fine Chem. Ltd.

2.7. Physicochemical evaluation

The powdered basidiome was used for the estimation of foreign matter, moisture content, ash values, extractive values [35], dry weight [36], flow characteristics (bulk density, tapped density, Carr's index, Hausner ratio), swelling index [37], dispersibility [38], absorption properties, foaming properties [39] and emulsion properties [40].

2.7.1. Foreign matter

The foreign matter attached to the surface of basidiome was separated before pulverization. The weighed powder of mushroom was observed for any foreign matter with naked eye as well as with the help of 6x lens. The percentage of foreign matter was calculated as follows:

$$\text{Foreign matter } (\%) = \frac{W2-W1}{W} \times 100$$

Where W1= weight of empty dish, W2= weight of dish with foreign matter, W= weight of sample.

2.7.2. Moisture content

The powdered sample (2 g) was taken in a tared glass petri dish and dried in an oven at 105°C until a constant weight was obtained. The samples were weighed after cooling in a desiccator to room temperature. Moisture content was calculated as below:

$$\text{Moisture content } (\%) = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

2.7.3. Dry weight

The dry weight was estimated by drying the mushroom powder in an oven at 105^0C for 24 h.

2.7.4. Flow characteristics

Approximately 50 g mushroom powder was taken in a 100 mL measuring cylinder. The volume covered initially by the powder in the measuring cylinder was recorded and the bulk density was estimated as ratio of initial weight of powder taken to volume V_B of powder in the measuring cylinder. The volume covered by the powder in the measuring cylinder after 500 manual taps was observed. The ratio of initial weight of powder taken and the volume V_T occupied after tapping was expressed as tapped density. The ratio of bulk density to tapped density was noted as Hausner ratio. A value of this ratio lower than 1.25 represents good flow properties and higher 1.25 indicates poor flow. Carr's index was determined using the relation Carr's index (C) = V_B-V_T/V_B×100

Where V_B = freely settled initial volume of a given weight of powder without tapping, V_T = tapped volume of same weight of powder after 500 manual taps. The value lower than 15% indicates good flow characteristics and a value greater than 25% indicates poor flow characteristics.

2.7.5. Dispersibility

Ground sample (5 g) was put into a 100 mL measuring cylinder and distilled water was added up to the mark of 100 mL. It was stirred and kept undisturbed for 1 h. The volume of settled particles was subtracted from 100 and the difference was reported as percentage dispersibility.

2.7.6. Ethanol soluble extractives

About (5 g) mushroom powder was macerated with 100 mL of 90% ethanol in a 250 mL flask. Then it was kept in orbital shaking incubator for 24 h followed by filtration. The filtrate (25 mL) was evaporated to dryness in a pre-weighed china dish. The residue was dried at 105°C and weighed. Percentage value of alcohol soluble extractive was measured with reference to the air-dried powdered sample.

2.7.7. Water soluble extractives

Five gram of mushroom powder was macerated with 100 mL of chloroform water in a 250 mL flask by keeping it in orbital shaking incubator for 24 h. The above mixture was filtered and the filtrate (25 mL) was allowed to evaporate in a pre-weighed china dish. The residue was dried at 105°C and weighed. Percentage value of water soluble extractive was estimated in relation to the air-dried powdered sample.

2.7.8. Oil absorption capacity

One gram powder of mushroom was mixed with 10 mL of refined soybean oil in a beaker. The mixture was stirred with a magnetic stirrer for 5 min. It was kept undisturbed at room temperature for 1 h. It was then centrifuged at 2000 rpm for 30 min and the supernatant was collected in a 10 mL graduated cylinder. Oil absorption capacity was estimated by subtracting the volume of oil added initially to the powder and volume of the supernatant collected after centrifugation. Oil absorption capacity was

represented as volume of oil absorbed per gram of dried powder.

2.7.9. Water absorption capacity

One gram mushroom powder was put in 10 mL distilled water in a beaker. The mixture was stirred using magnetic stirrer for 5 min. It was kept undisturbed for 1h at room temperature. The mixture was centrifuged at 2000 rpm for 30 min and the supernatant thus obtained was collected in a 10 mL graduated cylinder. Water absorption capacity was noted as volume of water absorbed per gram of dried powder.

2.7.10. Emulsifying capacity

Emulsion was prepared by taking 2g powdered sample in calibrated centrifuge tube followed by addition of 20 mL each of distilled water and refined soybean oil. Then centrifugation was done at 1600 rpm for 10 min. The emulsifying activity was calculated in percentage as the ratio of the height of the emulsified layer to the total height of the material in the tube.

2.7.11. Emulsion stability

Emulsion stability was estimated as percentage of the total height of the emulsified layer to the total height of the material in the tube after heating the tubes at 80^0C for 30 min followed by cooling and centrifugation at 1600 rpm for 15 min.

2.7.12. Total Ash value

Approximately (2 g) of powder was incinerated in a pre-weighed crucible at 450°C until free of carbon. It was weighed after cooling and the percentage total ash was estimated as

$$\text{Ash} \quad (\%) = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

Where W1= weight of empty crucible, W2= weight of crucible with ash, W = weight of sample.

2.7.13. Acid insoluble ash

The above obtained total ash was boiled for 5 min after pouring 25 mL of dilute HCl (2 M) in it. The insoluble ash was collected on an ashless filter paper and washed with 5 mL of hot water. It was then ignited in a pre-weighed crucible at a temperature not exceeding above 450°C until constant weight was arrived. After cooling and weighing the percentage of acid insoluble ash was calculated with relation to the air-dried powder.

$$\text{Acid insoluble ash} \quad (\%) = \frac{\text{W2} - \text{W1}}{\text{W}} \times 100$$

Where W1= weight of empty crucible, W2= weight of crucible with acid insoluble ash, W = weight of sample

2.7.14. Water soluble ash

The total ash made from basidiome following the procedure as described above was boiled with 25 mL of chloroform water for 5 min. The insoluble ash thus obtained after filtration was washed with 5 mL of hot water and ignited in a pre-weighed crucible at a temperature not elevating than 450°C

until constant weight was reached. It was then followed by cooling and weighing. The water soluble ash was obtained by subtracting the weight of the insoluble ash from that of the total ash. Then water soluble ash (%) was estimated with reference to the air-dried powder.

$$\text{Water soluble ash } (\%) = \frac{W2 - W1}{W} \times 100$$

Where W1= weight of total ash, W2= weight of water insoluble ash, W= weight of sample.

2.7.15. Foaming capacity

About 1 g mushroom powder was dispersed in 50 mL of distilled water. It was vigorously whipped for 30 min in a blender and then poured into a 100 mL graduated cylinder. The volume was recorded before and after whipping and the foaming capacity was calculated as percentage increase in volume.

2.7.16. Foaming stability

Foaming stability was calculated as a percentage of the initial foam volume obtained after whipping that remained stable after 30 min.

2.7.17. Swelling index

About 1 g powder was taken in 100 ml stoppered measuring cylinder. The initial volume (V_0) covered by the powder in the stoppered measuring cylinder was noted. Any increase in volume (V_t) occupied by the contents in the measuring cylinder after 24 h was observed. The swelling index was calculated by the following formula:

$$St = (V_t - V_0/V_t) \times 100$$

2.8. Preparation of extract

The dried powder of mushroom basidiome (150 g) was put in 1500 mL of 70% ethanol. The mixture was agitated in orbital shaking incubator at 80 rpm and 37^{0}C for 72 h followed by filtration. The filtrate was evaporated by distillation. The residue left was dried in a hot air oven at 45°C. The extract was then concentrated, dried and weighed. The yield of extract (%) was calculated. The organoleptic properties of the extract were recorded. The extract was preserved at -4^{0}C for future use. The steps followed for extraction are shown in Figure 1[41].

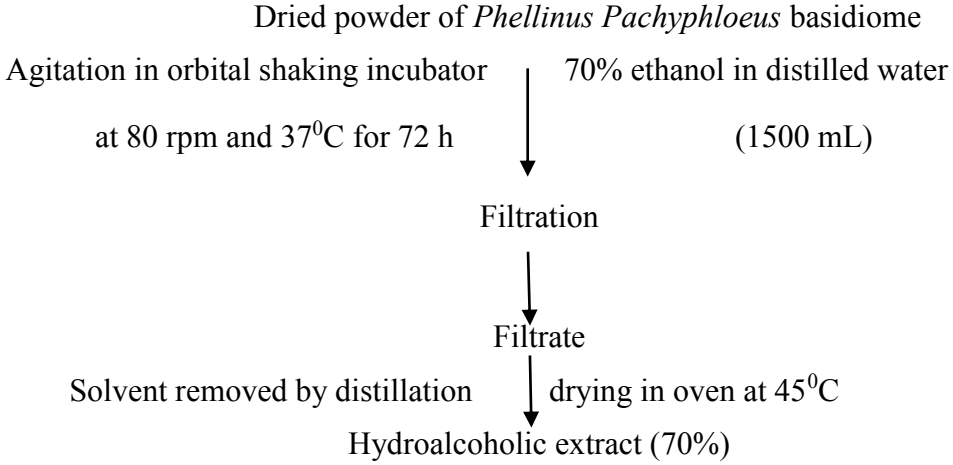

Fig. 1 Scheme followed for preparation of extract.

2.9. Mycochemical evaluation

The mycochemical examination of hydroalcoholic extract (70%) was performed following the established methods [42–46]

2.10. Statistical analysis

The results of physicochemical evaluation (n=3) and yield of extract (%) were expressed as mean ± standard error mean (SEM).

3. RESULTS AND DISCUSSION

3.1. Taxonomy

Table 1. Examined species and its origin (host and locality)

Species name	Herbarium number	Current name (www.mycobank.org)	Host	Locality	Collector names	Date of collection
***Phellinus pachyphloeus* (Pat.) Pat.**	PUN 5995	*Inonotus pachyphloeus* (Pat.) T. Wagner & M. Fisch.,	*Mangifera indica* L.,	Herbertpur, Dehradun, Uttarakhand (India)	Dhingra and Uzma Azeem	September 20, 2012

Detailed illustration of the examined species

1. *Phellinus pachyphloeus* (Pat.) Pat., Essai taxonomique sur les familles et les genres des *Hyménomycètes*: 97 (1900).

Figure 2.

Morphology of basidiome: Perennial, pileate, solitary, sessile, broadly attached, applanate, semicircular, ≤39 53× 28 cm*(L × W × T); upper surface olive brown to greyish brown to greyish black, glabrous, rimose, azonate to weakly zonate, crustose, crust ≤500 µm thick; lower surface light brown to brown when fresh, darkening and cracking on drying; pores round to angular, 5−6 per mm; dissepiments ≤74 µm thick; pore tubes ≤ 20 cm deep, light brown, stratified; context homogeneous, ≤7 cm at the base, very thin to inconspicuous in between the tubes; margins obtuse, irregularly wavy, concolourous on the upper surface, light brown, sterile ≤2.5 cm on the lower surface.

Microscopy of basidiome:

Generative hyphae: ≤2.6 µm wide, sybhyaline to pale yellow, branched, septate, thin-to-thick walled.

Skeletal hyphae: ≤6.5 µm wide, golden brown, occasionally branched, aseptate, thick-walled.

Hymenial setae: 32−98× 13−16.2 µm, subventricose to ventricose, acuminate, straight, dark brown, thick-walled; projecting ≤12.6 µm out of the hymenium.

Setal hyphae: ≤13.6 µm wide, with acuminate tips, dark brown, thick-walled.

Basidia not seen.

Basidiospores: 4.5−6.5 × 3.8−6 µm, broadly ellipsoid to subglobose, subhyaline to pale yellow, thick-walled, usually uniguttulate, inamyloid, acyanophilous.

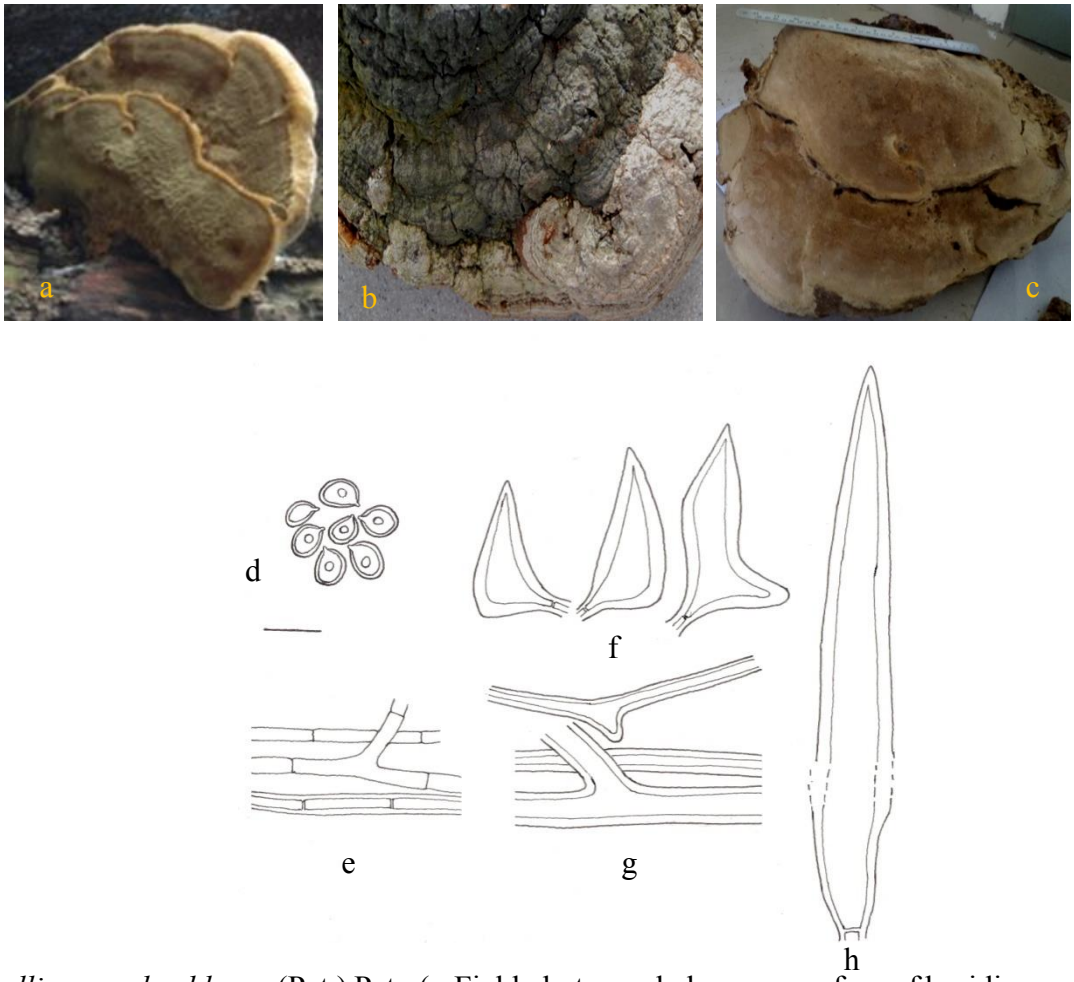

Fig 2. *Phellinus pachyphloeus* (Pat.) Pat.: (a-Field photograph, b-upper surface of basidiome, c-lower surface of basidiome, d-spores, e-generative hyphae, f-setae, g-skeletal hyphae, h-setal hypahe. Scale bar = 10 µm.

* (L = Length, W = Width and T = Thickness).

3.2. Physicochemical evaluation

The results of physicochemical evaluation were shown in Table 2.

Table 2. Physicochemical evaluation

Parameter	Value
Foreign matter (%)	0.01±0.01
Moisture (%)	13.67±2.33
Dry weight (%)	86.33±2.33
Bulk density (g/mL)	0.34±0.02
Tapped density (g/mL)	0.41±0.03
Carr's index (%)	14.04±0.83
Hausner ratio	1.16±0.01
Dispersibility (%)	85.67±1.21
Ethanol soluble extractives (%)	3.50±0.57

Water soluble extractives (%)	2.83±0.60
Oil absorption capacity (mL/g)	7.43±0.17
Water absorption capacity (mL/g)	7.90±0.10
Emulsifying capacity (%)	25.50±1.19
Emulsion stability (%)	22.63±0.81
Total ash (%)	3.50±0.28
Acid insoluble ash (%)	0.66±1.66
Water soluble ash (%)	3.0±0.57
Foaming capacity (%)	0.00±0.0
Foaming stability (%)	0.00±0.0
Swelling index (%)	0.00±0.0

Values are mean ± standard error mean; n = 3

3.3. Mycochemical screening

The powder made from basidiome of *Phellinus pachyphloeus*(Pat.) Pat. was used for the preparation of hydroalcoholic extract (70% ethanol). The yield of extract (%) was estimated and organoleptic properties were observed as given in Table 3. The extract was screened for various biochemical constituents. It showed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenols, flavonoids, tannins, anthraquinone glycosides, cardiac glycosides and alkaloids but lack cyanogenic glycosides, lipids, saponins and mucilages as shown in Table 4.

Table 3. Organoleptic properties

Species name	**Yield of extract (%) w/w, dry weight basis Mean ± SEM, n=3**	**Color**			**Odor**	**Consistency**
		Visible Light	**Short UV (254 nm)**	**Long UV (365 nm)**		
***Phellinus pachyphloeus* (Pat.) Pat.**	0.99 ± 0.15	reddish brown to dark brown	orange yellow	bright yellow	characteristic faint	sticky semisolid

Table 4. Mycochemical screening

Biochemical constituent/ chemical test	**Inference**
Carbohydrates	
Molisch's test	+
Anthrone test	+

Reducing sugars	
Fehling's test	+
Benedict's test	+
Proteins	
Xanthoproteic test	-
Lead acetate test	+
Million's test	+
Biuret test	+
Amino acids	
Ninhydrin test	-
Lead acetate	+
Steroids	
Hesse's test	-
Mole Schott's test	-
Salkowski's test	-
Liebermann-Burchard test	+
Triterpenoids	
Salkowski's test	+
Phenols	
Folin-Ciocalteu test	+
Ferric Chloride test	+
Flavonoids	
Shinoda test	+
Conc. Nitric acid test	+
Alkaline reagent test	+
Tannins	
Bramer's test	+
Lead acetate test	+
Potassium dichromate test	+
Glycosides	
Anthraquinone glycosides	
Borntrager's test	-
Modified Borntrager's	+

test	
Cardiac glycosides	
Baljet's test	+
Killer-Kiliani test	+
Cyanogenic glycosides	
Hydrogen cyanide test	-
Alkaloids	
Mayer's test	+
Wagner's test	+
Hager's test	+
Dragendorff's test	+
Fats and oils	
Saponification test	-
Sudan-III test	-
Saponins	
Froth test	-
Mucilages	
Ruthenium test	-
Swelling test	-
+= Preent; -=Absent.	

The details of morphology and microscopy of the specimen helped to identify it as *Phellinus pachyphloeus* (Pat.) Pat. The standardization of physicochemical properties is essential to determine the quality and purity of sample [47]. Foreign particles hinder the purity of the sample. So sample should be free from foreign matter. Foreign matter in the mushroom powder was found in much less quantity. High moisture content attracts microbial growth and elevates activity of enzymes causing degradation of intact basidiome and powder. Desirable low moisture content and high dry weight were observed in the mushroom sample. The powder showed good flow characteristics in terms of Carr's index being less than 1.25 and Hausner ratio being lower than 15%. Dispersibility of powder in water shows its reconstitutability. The mushroom powder showed good dispersibility value (85.67%) which was higher than reported for *Ganoderma* species [48]. The extractive values reveal the nature of chemical constituents of mushroom. The value of ethanol soluble extractives was more than water soluble extractives indicating the mushroom had more alcohol soluble polar constituents. Absorption properties give an idea whether the powder sample can be incorporated into aqueous or oily nutraceutical and drug formulations. Emulsion capacity and emulsion stability of powder makes it suitable to be used in commercial products like cosmetics, shampoos, pastes etc. Our study showed

good emulsion capacity and emulsion stability of *Phellinus pachyphloeus* powder. The mushroom powder did not show foaming properties and swelling which may be attributed to lack of saponins and mucilages in the sample. Ash values give an idea about the earthy material or inorganic compounds present in the powder. The values of total ash, acid insoluble ash and water soluble ash estimated for the powder lie in the range recorded for other mushrooms [49–50]. The results of mycochemical composition of *Phellinus pachyphloeus* were found in correlation with the earlier reports on mushrooms [51–55]. The presence of these primary and secondary metabolites indicates the high nutritional and medicinal value of *Phellinus pachyphloeus*.

4. CONCLUSION

We conclude that the work done in the present study will help in identification of *Phellinus pachyphloeus* and establishes standards to check adulteration of intact basidiome and powder available commercially. The results of preliminary mycochemical screening can prove helpful in finding the chemical constituents suitable for medicinal and nutraceutical applications which in turn may prove beneficial for human health.

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