Vol 9 | Issue 1| 2018 | 17-25.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

EVALUATION OF TAXONOMY, PHYSICOCHEMICAL PARAMETERS, AND MYCOCHEMICAL COMPOSITION OF WOOD DECAYING INDIAN FUNGI *Phellinus gilvus* (Schwein.) Pat. AND *Phellinus torulosus* (Pers.) Bourdot & Galzin: A COMPARATIVE STUDY

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ABSTRACT

The genus *Phellinus* is distributed to different ecogeographic zones causing different kinds of rots in living and dead angiosperms and gymnosperms. Many species of *Phellinus* are in use due to their nutraceutical and medicinal attributes all across the world since ages. However quality standards and biological activities are not studied for all species. This work aims at comparative evaluation of macroscopy, microscopy, physicochemical parameters, and mycochemical composition of hymenophores of *Phellinus gilvus* and *P. torulosus* collected from different localities of district Dehradun, Uttarakhand, India. *Phellinus gilvus* differs from *P. torulosus* in host, habit, external hymenophore characteristics, context, pore tubes, number of pores, margins, setae, shape and size of basidiospores. The hymenophore of each species was air-dried and pulverized to powder which was used for evaluation of physicochemical parameters, preparation of extract that in turn was used screening of mycochemical composition. Physicochemical analysis showed variation in relation to foreign matter, moisture content, dry matter, emulsion values, ash content, absorption properties, emulsion properties, dispersibility and flow properties. Mycochemical screening of hydroalcoholic extract (70% ethanol) showed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenolic compounds, flavonoids, tannins, anthraquinone glycosides, cardiac glycosides and alkaloids.

Keywords: Hymenophore, *Phellinus*, taxonomy, Uttarakhand.

INTRODUCTION

Phellinus Quél. is a cosmopolitan genus of family *Hymenochaetaceae*, order *Hymenochaetales*, class *Agaricomycetes*, sub-phylum, *Agaricomycotina* and phylum *Basidiomycota* [1]. Species of this genus have parasitic (trunk and roots) or saprophytic (logs, stumps and fallen branches) associations with both angiospermous as well as gymnospermous plants [2–5]. These cause degradation of lignin as well as cellulose and other related polysaccharides and are known to cause mainly white rot. In spite of great economic losses to forest flora caused by these fungi, their hymenophores have been used as folk medicine for the treatment of various diseases since antiquity [6–8]. *Phellinus* is reported to contain different bioactive compounds, such as carbohydrates, proteins, phenols, alkaloids, terpenoids, polysaccharides, steroids and fatty acids [9–12]. There are 95 species of this genus reported from India [13]. However physicochemical and chemical aspects have not been studied for all the species of this genus. In the present investigation, a comparative study of taxonomy, physicochemical parameters, and mycochemical screening were carried out on *Phellinus gilvus* and *P. torulosus* collected from Dehradun, Uttarakhand (India) with an endeavor to establish standards for their identity, quality, purity and chemical composition.

MATERIALS AND METHODS

Taxonomy

Collection of Fungi

The specimens were collected from Dehradun, Uttarakhand, India in the months of September and October during 2011–12. Field notes regarding host, habit of hymenophores, name of locality and macroscopic characteristics were made.

Macroscopy and microscopy

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Macroscopy of the hymenophore pertaining to upper and lower surfaces, type of context, type and number of pores, pore tubes, type of margins have been observed using a hand lens. The color standards used were in accordance with the Methuen's Handbook of colors [14]. Spore prints of each collection were taken for the detailed study of spores. Crush mounts and freehand sections in water and 5% KOH solution and staining in cotton blue (1%, in lactophenol), Congo red (1%, in distilled water), Phloxine (1%, in distilled water) and Melzer's reagent were made for microscopic examination comprising the structural details of basidiospores, basidia, hyphae and setae [15].

Submission of the specimen

The detailed illustration of each specimen was made on the basis of macroscopic and microscopic study and compared with the published literature. The specimens were identified as *Phellinus gilvus* and *P. torulosus*. Each specimen was assigned a unique herbarium number (PUN) and deposited in the herbarium of the Department of Botany, Punjabi University, Patiala, Punjab, India.

Chemicals

The chemicals used for taxonomy, extraction and mycochemical screening were of AR grade (Himedia, Merck India and SD Fine Chemical Ltd., India).

Physicochemical evaluation

The pulverized hymenophore of *Phellinus gilvus* and *P. torulosus* was used for the standardization of physicochemical parameters in triplicate. Foreign matter, moisture content, extractive values, ash values [16] dry matter [17], absorption properties, foaming properties [18], emulsion values [19], dispersibility [20], flow characteristics, swelling index [21] were determined.

Foreign matter

The sample powder (650 g) of each species was taken and the pieces of foreign matter were sorted out with naked eye and with the help of a lens (6×). All portions of foreign matter were pooled, weighed and percentage yield was calculated.

Moisture content

The sample powder (2 g) was taken and dried in the oven at 105°C until constant weight was achieved. Loss on drying was calculated as percentage of loss of water on drying.

Dry matter

This was taken as the final weight obtained after the sample have been dried in the oven at 105°C for 24 hours until no further change in weight was observed.

Extractive values

About 5 g powder was macerated with100 ml of 90% ethanol in a 250 ml flask for 24 h after a frequent shaking for 6 h. Then filtration was done and 25 ml of the filtrate was poured into the already weighed china dish. It was followed by evaporation to dryness, cooling and then weighing. Alcohol soluble extractive value in percentage w/w (on dry weight basis) was calculated with reference to the air dried mushroom powder taken initially. For the determination of water soluble extractive 100 ml of distilled water was used instead of alcohol.

Ash values

A 2 g quantity of powder was ignited in a pre-weighed crucible at temperature ≤450°C followed by cooling, weighing and then the percentage total ash was calculated. In case of acid insoluble ash, total ash thus obtained was boiled for 5 min with 25 ml of dilute HCl (2 M). The residue obtained after filtration was subjected to washing with 5 ml of hot water. It was then ignited in a pre-weighed crucible at a temperature ≤450°C to obtain constant weight. Acid insoluble ash (%) was calculated with reference to the air-dried mushroom powder. For the determination of water soluble ash content 25 ml of chloroform water was taken. The weight of the water insoluble ash subtracted from that of total ash provide the weight of the water soluble portion of total ash. The water soluble ash (%) was calculated with reference to the initial mushroom powder taken.

Absorption properties

One gram powder taken in 10 ml of distilled water or refined soybean oil was kept undisturbed for 1h at room temperature followed by centrifugation at 2000 rpm for 30 min. The supernatant was then taken in a 10 ml graduated cylinder. Water or oil absorption capacity was noted as volume of water or oil absorbed per gram of the dried powder sample.

Emulsion values

In a calibrated centrifuge tube 2 g powder was taken along with 20 ml each of distilled water and refined soybean oil for the formation of emulsion. Then centrifugation was done at 1600 rpm for 10 min. The emulsifying capacity (%) was calculated as the ratio of the height of the emulsified layer to the total height of the material in the tube. Emulsion stability (%) was estimated as the 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 cooled for 15 min and centrifugation was done at 1600 rpm for 15 min.

Dispersibility

Five gram of the mushroom powder was taken in a 100 ml measuring cylinder. Then distilled water was added up to the mark of 100 ml. It was kept undisturbed for 1h after stirring. The difference between the total volume i.e. 100 ml and the volume occupied by the settled particles in the measuring cylinder was given as percentage dispersibility.

Flow properties

About 50 g mushroom powder was put in a 100 ml measuring cylinder. The initial volume occupied by the powder in the measuring cylinder was noted and the bulk

density was obtained as ratio of initial weight to volume V_B of powder. After these 500 manual taps were done and the volume covered by the powder in the measuring cylinder was again noted. The ratio of initial weight of powder and the volume V_T noted after tapping was reported as tapped density. The ratio of bulk density to tapped density was calculated as Hausner ratio. A value below 1.25 represents good flow properties and above 1.25 indicates poor flow. Carr's index was calculated by the following relation Carrs 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.

Foaming properties

To 1 gram mushroom powder, 50 ml distilled water was put in a blender. It was then vigorously whipped for 30 min and poured into a 100 ml graduated cylinder. The volume of mixture before and after whipping was noted and foaming capacity (%) was calculated. The amount of foam that remained stable after 30 min was taken as foaming stability.

Swelling index

To note the swelling property, 1 g powder was weighed and taken in 100 ml stoppered measuring cylinder. The initial volume (V_0) and any increase in volume (V_t) occupied by the contents in the measuring cylinder after 24 h was recorded. The swelling capacity was calculated by the following formula

$St = (V_t – V_0/V_t) \times 100$

Preparation of extract

The dried mushroom powder (150 g) was extracted with 1500 ml of 70% ethanol [22] (Fig 1). The mixture was agitated for 72 h using orbital shaking incubator at 80rpm and 37^0C. The ethanol extract was filtered. The filtrate was then collected and the solvent was evaporated by simple distillation. The residue was dried in oven at 45°C. The residue was weighed and percentage yield was calculated in terms of the air-dried weight of the mushroom. The percentage yield was calculated and the sensory evaluation of extract was recorded as shown in (Table 2). The extract was stored at -4^0C until further use.

Mycochemical screening

The qualitative chemical examination of the extract was done following the standard methods [23–25].

Statistical analysis

The results of physicochemical evaluation (n=3) and yield of extract (%) were expressed as mean±standard error mean (SEM) were analyzed using the Student's t-distribution test of significance. Values were declared significant at $p < 0.05$.

RESULTS

Taxonomy

***Phellinus gilvus* (Schwein.) Pat., Essai Taxonomique sur les Familles et les Genres des *Hyménomycètes*.**

Figure 2 (a–g)

Macroscopy

Annual, pileate, woody hard, solitary to imbricate, applanate, somewhat convex, semicircular, ≤9.2 × 11.2 × 1.1 cm; upper surface yellowish brown to brownish orange towards the margins, reddish brown to dark brown towards the base, glabrous, usually azonate to sometimes zonate with wide zones, scrupose with irregular warts or protuberances, crustose, crust ≤500 µm thick; lower surface light brown to brown; pores round to angular, 10–11 per mm; entire, dissepiments ≤50 µm thick; pore tubes ≤4.5 mm deep, reddish brown to dark brown; context homogeneous, ≤6 mm thick, yellowish brown to pale reddish brown; margins acute, somewhat wavy, inturned on maturity, brownish orange on the upper surface, light brown, sterile ≤1 mm on the lower surface.

Microscopy

Generative hyphae ≤3.2 µm wide, branched, aseptate, thick-walled.

Skeletal hyphae ≤5.2 µm wide, golden brown to yellowish brown, unbranched, aseptate, thick-walled.

Hymenial setae 30−36 × 9.7−11.1 µm, subulate, acuminate, straight, dark brown to rusty brown, thick-walled; projecting ≤17 µm out of the hymenium.

Tramal setae and setal hyphae absent.

Basidia 7.6−14.3 × 3.4−5.8 µm, clavate; sterigmata ≤3.2 µm long.

Basidiospores 4.5−6.5 × 3.2−4 µm, broadly ellipsoid to subglobose, subhyaline to pale yellow, thin-walled, acyanophilous.

Collections examined: Lachhiwala, on trunk of *Shorea robusta*, Uzma Azeem 5997 (PUN), October 12, 2011.

***Phellinus torulosus* (Pers.) Bourdot & Galzin, Bulletin de la Société Mycologique.**

Figure 2 (h–n)

Macroscopy

Perennial, pileate, imbricate, broadly attached, applanate, ≤9.3 × 10.4 × 5 cm; upper surface reddish brown to dark brown, irregularly sulcate, crustose, crust ≤500 µm; lower surface light brown to brown, pores round to angular, 5−7 per mm; dissepiments ≤67 µm thick; pore tubes ≤17 mm deep, greyish brown to rusty brown, conclorous with the pore surface, stratified; context homogeneous, ≤23 mm thick, reddish brown to dark brown, ≤500 thick between the tubes; margins obtuse, irregularly wavy to lobed, concolourous on the upper surface, light brown, sterile ≤6 mm on the lower surface.

Microscopy

Generative hyphae ≤2.6 µm wide, branched, aseptate, thick-walled. Skeletal hyphae ≤.4 µm wide, golden yellow, unbranched, aseptate, thick-walled. Hymenial setae 25−43 × 7.7−9.7 µm, subulate to slightly ventricose, acuminate,

thick-walled, dark brown; projecting ≤21 µm out of the hymenium.

Tramal setae and setal hyphae absent. Basidia 9.7−12.3 × 5.2−6.5 µm, clavate; sterigmata ≤3.2 µm long. Basidiospores 3.8−5.2 × 2.6−4 µm, broadly ellipsoid to subglobose, subhyaline to pale yellow, thin-walled, weakly cyanophilous.

Collections examined: Dehradun-Mussoorie road, on trunk of *Melia azedarach*, Uzma Azeem 5994 (PUN), September 20, 2012.

Physicochemical parameters

The standardization of physicochemical parameters was done and results obtained were as shown in Table 1.

Preparation of extract

The crude hydroalcoholic (70% ethanol) extract of the each selected *Phellinus* species was prepared from powder of hymenophore and percentage yield of extract was calculated (Table 2).

Yield of extract and Sensory evaluation

The observations regarding yield (%) and sensory evaluation of each mushroom extract were as shown in Table 3.

Mycochemical screening

The mycochemical screening of hydroalcoholic (70% ethanol) extract of each *Phellinus* mushrooms was observed as in Table 4. The test extracts of both the species were found consisting of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenolic compounds, flavonoids, tannins, anthraquinone glycosides, cardiac glycosides and alkaloids but lacking cyanogenic glycosides, lipids, saponins and mucilages.

Table 1. Physicochemical evaluation of mushroom samples

Parameter	*P. gilvus*	*P. torulosus*
Foreign matter (%)	0.16±0.02[a]	0.02±0.01[b]
Moisture content (%)	21.33±3.87[a]	11±1.73[a]
Dry matter (%)	78.67±3.87[a]	89±1.73[a]
Extractive values **(%)**		
Ethanol soluble extractives	2.93±0.35[a]	2.33±0.66[a]
Water soluble extractives	1.73±0.35[a]	1.83±0.44[a]
Ash content (%)		
Total ash	4.33±0.16[a]	3.83±0.33[a]
Acid insoluble ash	1.66±0.44[a]	1.5±0.33[a]
Water soluble ash	1.5±0.57[a]	1.08±0.22[b]
Absorption properties (ml/g)		
Oil absorption capacity	6.78±0.64[a]	7.16±0.12[a]
Water absorption capacity	6.16±0.21[a]	7.16±0.17[b]
Emulsion properties (%)		
Emulsifying capacity	28.62±0.91	31.58±0.87
Emulsion stability	21.35±0.59[a]	27.62±1.49[b]
Dispersibility (%)	83.67±0.88[a]	84.33±1.33[a]
Flow properties		
Bulk density (g/ml)	0.14±0.01[a]	0.19±0.02[a]
Tapped density (g/ml)	0.18±0.01[a]	0.22±0.23[a]
Carr's index (%)	23.37±3.70[a]	11.87±1.04[a]
Hausner ratio	1.31±0.06[a]	1.12±0.02[a]
Foaming properties (%)		
Foaming capacity	0.00	0.00
Foaming stability	0.00	0.00
Swelling Index (%)	0.00	0.00

Values are mean ±standard error; n=3. Values in the same row with the different superscript letter are significantly different ($p < 0.05$).

Table 2. Yield of extract and sensory evaluation

Name of the Species	Color			Odor	Consistency	Yield (%) w/w, dry weight basis (Mean±SEM; n=3)
	Visible Light	Short UV (254 nm)	Long UV (365 nm)			
P. gilvus	reddish brown to dark brown	orange yellow	bright yellow	characteristic faint	sticky semisolid	1.44±0.33

P. torulosus	reddish brown to dark brown	orange yellow	bright yellow	characteristic faint	sticky semisolid	0.93±0.32

UV-Ultraviolet

Table 3. Mycochemical screening

Biochemical constituent/ chemical test	*P. gilvus*	*P. torulosus*
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	-	-
+ Present; - Absent		

Fig 1. Preparation of extract

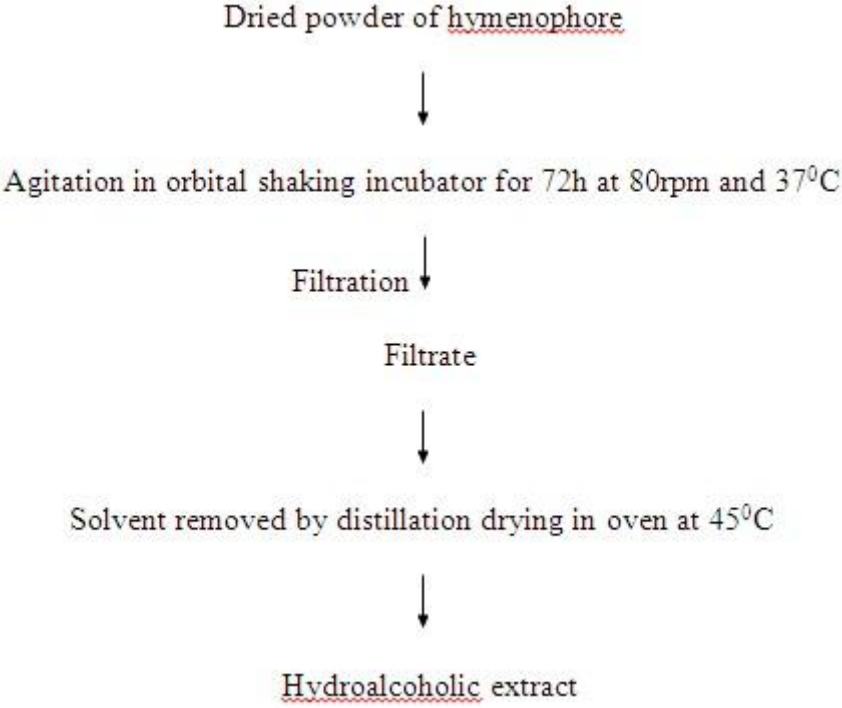
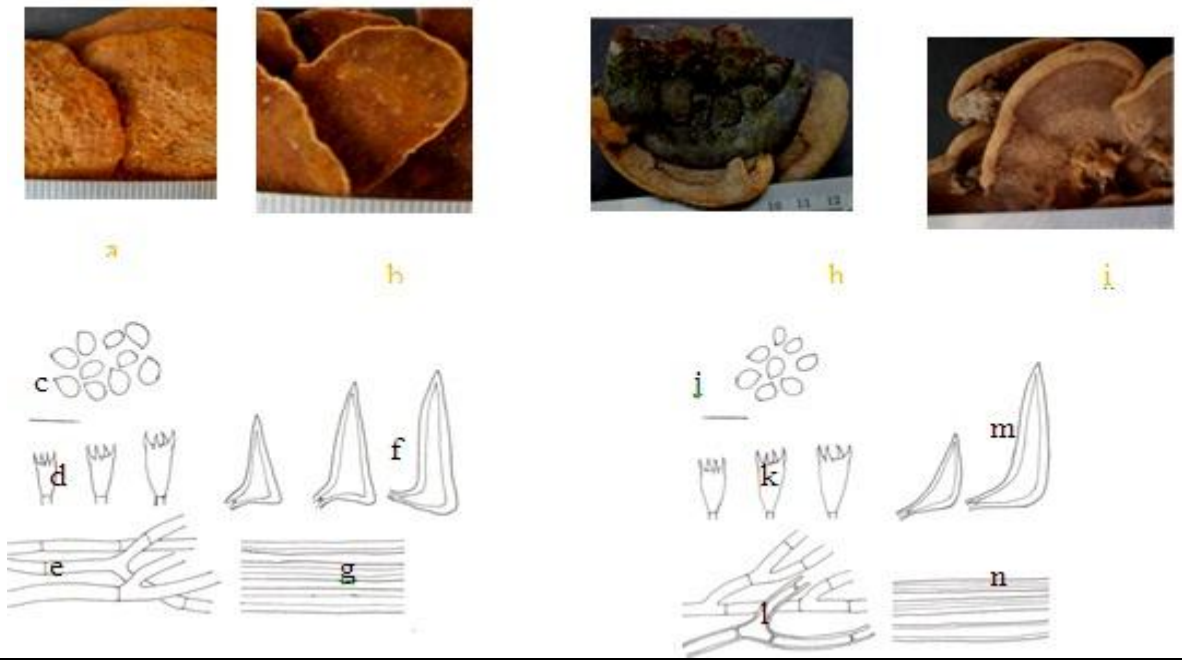


Fig 2. *Phellinus gilvus* (a-upper surface of hymeophore, b-lower surface of hymeophore, c-spores, d-basidia, e-generative hyphae, f-setae, g-skeletal hyphae); *P. torulosus* (h-upper surface of hymeophore, i-lower surface of hymeophore, j-spores, k-basidia, l-generative hyphae, m-setae, n-skeletal hyphae); scale bar = 10 µm.



DISCUSSION

It is mandatory to ensure the identity, quality and purity of natural products before their utilization in therapeutic and nutraceutical formulations. Macroscopy and microscopy of the source material is a prerequisite for establishing its correct identification. *Phellinus gilvus* can be distinguished from *P. torulosus* primarily in having annual hymenophore with acute margins, scrupose upper surface, more number of pores (10–11), smaller setae, and longer and acyanophilous spores. Foreign matter present in a sample or drug mask its quality and purity. Therefore it cannot be neglected. A very small amount of foreign

matter was collected from both the samples. It lies in the range noted for other mushrooms [26–27]. The higher the moisture content, the greater is the degradation of the mushroom sample due to enhanced microbial growth and hydrolytic enzyme activity. The moisture content in the investigated mushrooms (11–21.33%) is low and is comparable to that reported for other mushrooms [28–29]. Dry matter (78.67–89%) was greater as compared to *Auricularia polytricha* (9.4%) and *Pleurotus ostreatus* (6.7%) but lower than (93.7−98.33%) reported for *Lentinus squarrosulus* and *Psathyrella atroumbonata* [30–31]. Extractive values indicate that each tested mushroom had greater alcohol soluble polar constituents than water soluble constituents. Ash content provides an indication of earthy material or inorganic compounds in the drug. The ash content of mushrooms was found in the range 1.3–6.3% estimated for *Phellinus linteus* [32]. Water soluble ash indicates the amount of inorganic constituents in herbal drugs. Acid insoluble ash gives an idea of silica present and contamination with earthy material. The values for acid insoluble ash ranged from 1.5–1.66% and for water soluble ash from 1.0–1.5%. Imbibition of water is an important trait in food products such as sausages, custards and doughs. Water absorption depends on amount and type of hydrophilic constituents, pH and nature of the powder [33]. Oil absorption capacity indicates the rate at which proteins bind to fats in food and drug formulations [34]. *Phellinus torulosus* had higher oil absorption capacity and water absorption capacity than *P. gilvus*. Emulsion capacity is the ability of powder to emulsify oil. Emulsions play a crucial role in pharmaceutical preparations like cosmetics, pastes etc. Emulsions have also been used for treating skin disorders, lacerations and for drug delivery etc. [35]. Certain biochemical constituents too help in stabilizing the emulsion [36]. *Phellinus torulosus* showed greater emulsion capacity and emulsion stability. Bulk density is a measure of heaviness of powder which provides the relative volume of the packaging material required. Dispersibility of powder in water gives an idea of its reconstitutability. Both the studied mushrooms showed good values for bulk density (0.14–0.19 g/ml) and dispersibility (83.67–84.33%). Flow properties indicate that powder may be utilized as a direct compression excipient. Hausner ratio provides interparticle friction and Carr's index is a measure of compressibility of powder. Hausner ratio and Carr's index of *Phellinus torulosus* was observed lower than 1.25 and l5% respectively indicating good flow properties. Foaming capacity is the ability of a powder to form foam. It is related to the amount of solubilized proteins [37] and polar and non-polar lipids in the sample [38]. Saponins also play a role in foam formation. Foams are used to improve texture, consistency and appearance of food and drug [39]. There was observed no foam formation in any of the tested mushroom samples. The powder of any tested *Phellinus* mushroom did not swell indicating lack of mucilage substances. The sensory evaluation provides useful information which may prove helpful in authentication and detection of adulteration for quality control. The results of mycochemical analysis of each species are in correlation with literature reports on mushrooms [40, 41, 42, 43].

CONCLUSIONS

The aggregate data in this investigation establishes standards which may prove beneficial to identify these genuine species and to check purity in the intact hymenophore as well as powder of each species available commercially. These contribute directly or indirectly to the safety, effectiveness and acceptability of the product which can be used either as good source for nutraceutical and pharmaceutical preparations and the mycochemical analysis is helpful in finding the chemical constituents that may have medicinal properties and can be employed for the management of various disorders. The results of the present study may be used in future for authentication and quality control of different species of *Phellinus*.

ACKNOWLEDGEMENT

This work was financially supported by UGC, New Delhi, for MANF (Maulana Azad National Fellowship) granted to the corresponding author during 2011–2016. The authors graciously acknowledge Head, Department of Botany, Punjabi University, Patiala for providing the necessary laboratory facilities to accomplish this research work.

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