Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2151-2158
Received: 04-11-2017
Accepted: 05-12-2017

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Comparative analysis of taxonomy, physicochemical characteristics and mycochemical screening of two wood degrading *Phellinus* mushrooms (*P. fastuosus* (Lév.) S. Ahmad and *P. sanfordii* (Lloyd) Ryvarden)

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Abstract
Phellinus mushrooms are of great interest for their nutritive and medicinal properties. Indian fungal flora is rich in economically important *Phellinus* species. Of these some are in use against various human ailments since ages. However physicochemical characterization and biochemical composition of all the *Phellinus* species has not been evaluated. In the present investigation, *Phellinus fastuosus* and *P. sanfordii* were evaluated for taxonomy, physicochemical characteristics and mycochemical composition of hydroalcoholic (70% ethanol) extract. Both the tested mushrooms showed comparable foreign matter, moisture content, dry weight, bulk density, tapped density, Carr's index, Hausner ratio, ethanol soluble extractives, water soluble extractives and ash values. The values of oil absorption capacity, water absorption capacity, emulsion capacity and emulsion stability vary significantly among the tested mushrooms. The two tested mushrooms did not possess foaming and swelling properties. This information can prove beneficial for future utilization of *Phellinus fastuosus* and *P. sanfordii* in nutraceutical and pharmaceutical products. The mycochemical screening showed that each tested mushroom extract consisted of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenolic compounds, flavonoids, tannins, anthraquinone glycosides, cardiac glycosides and alkaloids.

Keywords: Basidiome, *Phellinus*, Taxonomy, Uttarakhand

Introduction
A number of pharmacological properties have been credited to mushrooms. Of these mushrooms *Phellinus* Quél. (*Hymenochaetaceae*) constitute a good source of pharmaceuticals and healthcare products. There are 360 records of *Phellinus* worldwide [1]. These represent wood inhabiting macrofungi causing white rot of a wide range of angiosperms and gymnosperms [2–4]. In India 97 species of this genus have been reported [5–7]. The history of *Phellinus* species used as folk medicine is probably very old as these are mentioned in ancient medicinal literature [8]. *Phellinus linteus* has been mentioned in many Chinese medical books, as the world's earliest pharmacopoeia issued by the Tang government "New compendium of Materia Medica" [9], "Chinese Compendium of Materia Medica" of Shi-Zhen Li in the Ming Dynasty [10] and "Flora Fungorum Sinicorum" [11]. Various species of *Phellinus* such as *P. rimosus, P. conchatus, P. baumii, P. igniarius, P. nigricans* and *P. senex* have been used as traditional medicines [12–15]. In India, *Phellinus rimosus* has been used for the treatment of mumps by the tribal people in Kerala [16] and *P. durissimus* has been employed in traditional medicinal practices by the tribes of Dang district of South Gujarat [17]. The folk medicinal use of *Phellinus* species has been traced to the extraction and mycochemical characterization of these mushrooms. A progressive research on *Phellinus* species witnesses various bioactivities of the crude extract of these species such as anti-inflammatory [18–19], hepatoprotective [20], antidiabetic [21–23], anticancer [24–25], antioxidant [26–29], immuno-stimulatory [30], cyto-protective [31] and anti-microbial activities [32–34]. These mushrooms are a natural cocktail of a variety of secondary metabolites responsible for these activities [35–38]. Mushrooms are becoming popular for drug development due to the belief that natural medicine is safe, affordable and imposes less side effects. Therefore identification, physicochemical characterization and knowledge of the mycochemical constituents of mushrooms is desirable. This information can prove beneficial for the development of various pharmaceutical and nutraceutical formulations. The present investigation has been selected to work out the taxonomy, determine the physicochemical properties and screen the biochemical constituents of *Phellinus fastuosus* and *P. sanfordii*.

2. Materials and methods

2.1. Collection of mushrooms

Phellinus fastuosus and *P. sanfordii* were collected from different localities of district Dehradun (Uttrakhand), India.

2.2. Morphology

Morphological details of color, type and consistency of basidiome (pilear, hymenial surface and margins) were studied. The dimensions, color, type of context, tube layers, and type and number of pores per mm were examined. The color standards were compared with Methuen's Handbook of colors [39]. Spore prints of fresh specimens were taken for the detailed study of spores. The basidiomes were air dried at room temperature. The collections were then kept in cellophane packets/zip lock polythene with 1, 3-Dichlorobenzene crystals.

2.3. Micro-morphology

Microscopic details of each collection pertaining to type, color, dimensions of hyphae, basidia, basidiospores, setae and setal hyphae were observed by making crush mounts and cutting free hand sections in water as well as 3%/5%/10% KOH solutions followed by their staining in 1% Phloxine and 1% Congo red. The color reactions (amyloid and cyanophilous) of spores were observed using Melzer's reagent (0.5 g Iodine, 1.5 g Potassium iodide, 20 g chloral hydrate and 20 mL distilled water) and 1% cotton blue in lactophenol respectively. Line diagrams of microscopic structures were drawn using compound light microscope at different magnifications 10x × 10x, 10x × 40x, 10x × 100x (oil immersion lens) and camera lucida.

2.4. Documentation and submission of specimens

A detailed description pertaining to morphology and micro-morphology of each specimen was made. It was followed by comparison of each description with published literature and physical comparison with the type material lying at the herbarium, Department of Botany, Punjabi University, Patiala and Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg (Sweden). Each specimen after identification as *Phellinus fastuosus* and *P. sanfordii* and assigning a respective herbarium number (PUN) was submitted at the internationally recognized herbarium of Department of Botany, Punjabi University, Patiala.

2.5. Chemicals and reagents

The chemicals used for taxonomic study, extraction and mycochemical screening were of AR grade (Himedia, Loba chemie and SD Fine Chemical Ltd., India).

2.6. Physicochemical properties

The powdered basidiome of *Phellinus fastuosus* and *P. sanfordii* was used for the determination of physicochemical parameters in triplicate. The estimation of foreign matter, moisture content, ash values and extractive values [40], dry weight [41], flow characteristics (bulk density, tapped density, Carr's index, Hausner ration) and swelling index [42], dispersibility [43], absorption properties, foaming properties [44] and emulsion properties [45].

2.6.1. Foreign matter

The foreign matter adhered to the specimen was detached before crushing it into powder. Approximately (650 g) powder of mushroom was examined for any the presence of any foreign material with naked eye as well as with the help of 6x lens. The foreign matter (%) was calculated as below

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

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

2.6.2. Moisture content

The powder of each mushroom (2 g) was taken in a tared glass pre-weighed petri dish separately and was allowed to dry in an oven at 105°C until a constant weight was reached. Then each powder sample was weighed after cooling it in a desiccator to room temperature. Moisture content was recorded by the following formula

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

2.6.3. Dry weight

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

2.6.4. Flow characteristics

Mushroom powder (50 g) was taken in a 100 ml measuring cylinder. The initial volume of powder in the measuring cylinder was noted down and the bulk density was calculated as ratio of initial weight of powder taken to volume V_B covered by the powder in the measuring cylinder. The volume of powder in the measuring cylinder after 500 manual taps was penned down. The ratio of initial weight of powder taken and the volume V_T of powder in the measuring cylinder after 500 manual taps was considered as tapped density. The ratio of bulk density to tapped density was represented as Hausner ratio. Carr's index was determined using the equation 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.

2.6.5. Dispersibility

The mushroom powder (5 g) was taken in a 100 ml measuring cylinder and distilled water was poured up to the mark of 100 ml. It was mixed well by stirring and then allowed to stand for 1 h. The volume of settled particles in the measuring cylinder was subtracted from 100 and the difference was reported as percentage dispersibility.

2.6.6. Ethanol soluble extractives

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

2.6.7. Water soluble extractives

The procedure for the estimation of water soluble extractives (%) was same. The only difference is that chloroform water was used in place of 90% ethanol for extraction.

2.6.8. Oil absorption capacity

Mushroom powder (1 g) was mixed with 10 ml of refined soybean oil in a beaker. This mixture after stirring with a magnetic stirrer for 5 min was allowed to stand at room temperature for 1 h. It was followed by centrifugation at 2000 rpm for 30 min. The supernatant was collected in a 10 ml graduated cylinder. Oil absorption capacity was determined by subtracting the volume of oil poured 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.

2.6.9. Water absorption capacity
The same procedure was followed to estimate the water absorption capacity of mushroom powder. The difference is that 10 ml distilled water was used instead of refined soybean oil.

2.6.10. Emulsifying activity
About 2 g mushroom powder was taken in a calibrated centrifuge tube. Then 20 ml each of distilled water and refined soybean oil was added. It was followed by centrifugation 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 covered by the material in the tube.

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

2.6.12. Total Ash value
About (2 g) mushroom powder was incinerated in a pre-weighed crucible at 450°C until free of carbon. The contents were allowed to cool at room temperature. The percentage total ash was estimated by the following equation

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

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

2.6.13. Acid insoluble ash
The above amount of total ash was boiled for 5 min in 25 ml of dilute HCl (2 M). After filtration, the insoluble ash was collected on ashless filter paper. It was washed with 5 ml of hot water and was ignited in a pre-weighed crucible at a temperature not exceeding above 450°C until constant weight was observed. The crucible was allowed to cool and followed by weighing. The acid insoluble ash was calculated with relation to the air-dried powder as follows.

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

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

2.6.14. Water soluble ash
The same protocol was used as followed for the estimation of acid insoluble ash except that 25 ml of chloroform water for was used in place of 25 ml of dilute HCl (2 M).

2.6.15. Foaming capacity
Approximately (1 g) powder sample was put in a blender and 50 ml of distilled water was added. It was whipped well for 30 min in the blender and then poured into a 100 ml measuring cylinder. The volume occupied by the contents was noted before and after whipping and the foaming capacity was calculated as percentage increase in volume.

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

2.6.17. Swelling index
The mushroom powder (1 g) was taken in a 100 ml stoppered measuring cylinder. The initial volume (V_0) of the powder in the stoppered measuring cylinder was penned down. Any increase in volume (V_t) covered by the contents in the measuring cylinder after 24 h was noticed. The swelling index was estimated as follows

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

2.7. Yield of extraction and sensory evaluation
The dried powder of each mushroom basidiome (150 g) was extracted with 1500 ml of 70% ethanol. The mixture was agitated for 72 h using orbital shaking incubator at 80 rpm and 37^0C followed by filtration. The filtrate was subjected to evaporation by distillation. The residue was kept to dry in a hot air oven at a temperature of 45°C. The obtained extract of each mushroom was then dried in air tight desiccator and kept in deep freezer at -4^0C for future use [46].

2.8. Mycochemical screening
The qualitative chemical tests of hydroalcoholic extract (70% ethanol) of both the mushrooms were performed following the standard methods [47–51].

2.9. Statistical analysis
The results of physicochemical properties (n=3) and yield of extract (%) were represented as mean ± standard error mean (SEM) and were analyzed using the Student's t-distribution test of significance. Values were said to be significant at $p < 0.05$.

3. Results
3.1. Taxonomy of mushrooms
1. *Phellinus fastuosus* (Lév.) S. Ahmad, *Basidiomycetes* of West Pakistan: 56, 1972.
Figure 1.

Morphology of basidiome: Perennial, pileate, woody hard, solitary to imbricate; broadly attached, ≤10 × 11 × 2.5 cm (L × W × T)*; abhymenium greyish brown to dark brown, glabrous, concentrically sulcate, zonate, crustose, crust ≤480 µm thick; hymenium surface light brown to brown, glancing; pores round to angular, 7−10 per mm; dissepiments ≤57 µm thick; pore tubes ≤10 mm deep, conclorous with the pore surface, stratified; context homogeneous, ≤13 mm thick, yellowish brown to brown, ≤1 mm thick in between the tubes; margins obtuse, irregular to somewhat wavy, concolourous on the abhymenial surface, sterile ≤3 mm, paler concolourous on the hymenial surface.

Micro-morphology of basidiome: Hyphal system: Dimitic. Generative hyphae: ≤3 µm wide, subhyaline to plae yellow,

branched, septate, thin-walled. Skeletal hyphae: ≤5.2 µm wide, golden brown to rusty brown, unbranched, aseptate, thick-walled. Setae and setal hyphae absent. Basidia 7.7−12.3 × 4.5−5.8 µm, clavate; sterigmata ≤3.2 µm long. Basidiospores: 5.2−7.2 × 3.8−5.2 µm, broadly ellipsoid to ovoid to subglobose to globose, golden yellow to golden brown to rusty brown, thick-walled, inamyloid, usually uniguttulate, acyanophilous. Material examined: Rajpur, on trunk of *Ficus religiosa* L., Uzma Azeem 5993 (PUN), October 5, 2012.

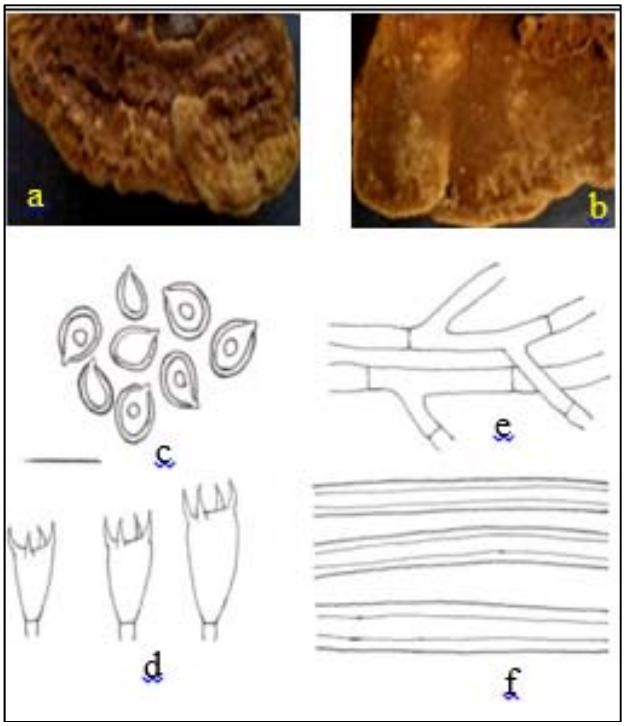

Fig 1: a. Abhymenial surface, b. Hymenial surface, c. Basidiospores, d. Basidia, e. Generative hyphae and f. Skeletal hyphae; Scale bar = 10 µm

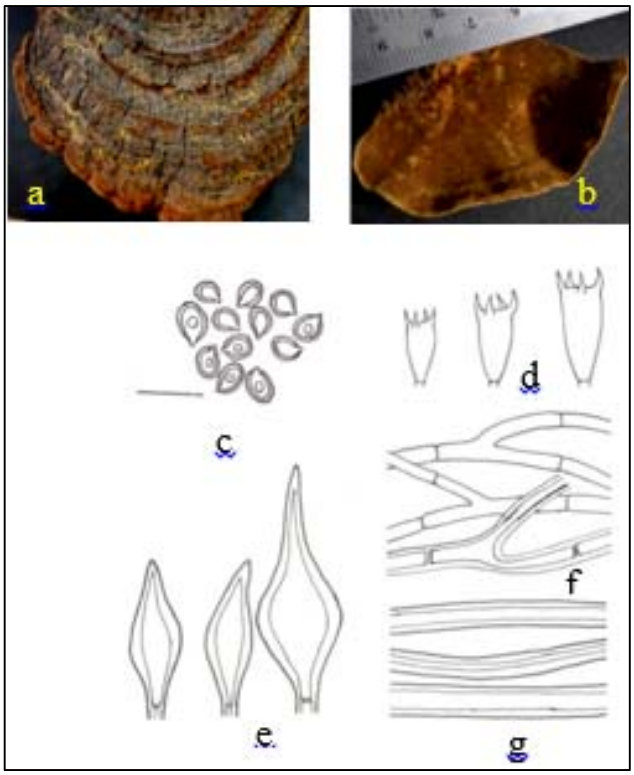

Fig 2: a. Abhymenial surface, b. Hymenial surface, c. Basidiospores, d. Basidia, e. Setae, f. Generative hyphae and g. Skeletal hyphae; Scale bar =10 µm

2. *Phellinus sanfordii* (Lloyd) Ryvarden, Norwegian Journal of Botany 19: 235, 1972.

Morphology of basidiome: Perennial, pileate, woody hard, imbricate, broadly attached, ≤5.3 × 9.2 × 2 cm (L × W × T)*; abhymenium brown to reddish brown to dark brown, tomentose to glabrous, radially rimose, sulcate, zonate, crustose, crust ≤500 µm thick; hymenium light brown to reddish brown to dark brown becoming paler towards the margins, glancing; pores round to angular, 6−8 per mm; dissepiments ≤53 µm thick; pore tubes ≤14 mm deep, conclorous with the pore surface, stratified; context homogeneous, ≤4 mm thick, reddish brown to dark brown, ≤500 µm thick between the tubes; margins acute, irregularly wavy, conclorous on the abhymenial surface, light brown, sterile ≤2 mm on the hymenial surface.

Micro-morphology of basidiome: Hyphal system: Dimitic. Generative hyphae ≤2.6 µm wide, subhyaline to plae yellow, branched, septate, thin to thick-walled. Skeletal hyphae ≤3.8 µm wide, golden brown, unbranched, aseptate, thick-walled. Hymenial setae 15−35×6.5−12.3 µm, subventricose to ventricose, acuminate, thick-walled, dark brown, projecting ≤17 µm out of the hymenium. Tramal setae and setal hyphae absent. Basidia 7.7−9.1×4.5−5.8 µm, broadly clavate and sterigmata ≤2.6 µm long. Basidiospores 3.8−6.5×2.6−4.5 µm, broadly ellipsoid to ovoid to subglobose, subhyaline to pale yellow to golden yellow, thick-walled, inamyloid, acyanophilous to weakly cyanophilous. Material examined: Mussoorie to Dhanaulti road, on trunk of *Desmodium gangeticum* (L.) DC., Dhingra and Uzma Azeem 8809 (PUN), April 29, 2014.

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

3.2. Physicochemical properties

The evaluation of physicochemical properties was done and the results are given in Table 1.

Table 1: Physicochemical evaluation of mushroom samples

Parameter	*P. fastuosus*	*P. sanfordii*
Foreign matter (%)	0.04±0.03[a]	0.06±0.04[a]
Moisture (%)	16.67±3.71[a]	23.83±2.62[a]
Dry weight (%)	83.33±3.71[a]	76.17±2.62[a]
Bulk density (g/ml)	0.29±0.02[a]	0.37±0.01[a]
Tapped density (g/ml)	0.34±0.02[a]	0.44±0.01[a]
Carr's index (%)	15.27±2.37[a]	16.74±0.81[a]
Hausner ratio	1.18±0.03[a]	1.17±0.01[a]
Dispersibility (%)	81±1.53[a]	93.33±0.33[b]
Ethanol soluble extractives (%)	4.33±1.02[a]	2.13±0.48[a]
Water soluble extractives (%)	2.5±0.28[a]	2.8±0.23[a]
Oil absorption capacity (ml/g)	5.03±0.08[a]	7.1±0.05[b]
Water absorption capacity (ml/g)	5.93±0.14[a]	4.56±0.14[b]
Emulsifying activity (%)	37.58±1.03[a]	21.82±0.63[b]
Emulsion stability (%)	31.16±1.12[a]	18.33±0.63[b]
Total ash (%)	5.16±0.44[a]	3.33±0.60[a]
Acid insoluble ash (%)	1.33±0.28[a]	1.16±0.44[a]
Water soluble ash (%)	2±0.57[a]	1.66±0.33[a]
Foaming capacity (%)	0.00	0.00
Foaming stability (%)	0.00	0.00
Swelling index (%)	0.00	0.00

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

3.3. Yield of extraction and sensory evaluation: The yield of extract (%) and sensory evaluation are shown in Table 2.

Table 2: Yield (%) of extraction and sensory evaluation

Mushroom	Extraction yield (%)	Color			Odor	Consistency
		Visible Light	Short UV (254 nm)	Long UV (365 nm)		
P. fastuosus	2.97±0.42	reddish brown to dark brown	orange yellow	bright yellow	characteristic faint	sticky semisolid
P. sanfordii	1.24±0.17	reddish brown to dark brown	orange yellow	bright yellow	characteristic faint	sticky semisolid

Values are mean ± standard error mean; n = 3.

3.4. Mycochemical screening: The biochemical analysis of hydroalcoholic (70% ethanol) extract of each tested *Phellinus* mushroom was done to detect the presence or absence of various biochemical constituents as shown in Table 3. The test extract of each 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 3: Qualitative biochemical screening

Biochemical constituent/ chemical test	*P. fastuosus*	*P. sanfordii*
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	-	-
+ = Positive ; - = Negative		

4. Discussion

The study of taxonomy, physicochemical characteristics and mycochemical composition of natural products is prerequisite in pharmacognostic evaluation. The comparative evaluation of taxonomy in the present investigation showed that *Phellinus fastuosus* can be distinguished from *P. sanfordii* in various morphological characters such as size of basidiome, number of pores, thickness of dissepiments and context, tube length and type of margins. Microscopically, *Phellinus fastuosus* lacked setal elements while *P. sanfordii* consisted of hymenial seate. The findings regarding the physicochemical properties showed low values of foreign matter and moisture content indicating good quality of these mushrooms. High foreign matter and moisture content impairs the purity of the sample making it undesirable for use. Young mushroom samples showed high moisture content and low dry weight as compared to mature fruiting bodies [52–53]. As per literature reports, the moisture content of wild mushrooms ranged from 26.2–90.26% [54] and dry matter from 91.25–95.54% [55]. The value of bulk density is a measure of the heaviness of powdered sample. The higher is the bulk density of a powder, the greater is its dispersibility. Both the tested mushrooms showed good values of bulk density and dispersibility which are comparable to the values reported for *Ganoderma* species [56–57]. Carr's index represents compressibility of powder while Hausner ratio is interparticle friction. The value of Carr's index less than 15% and Hausner ratio below 1.25 indicate good flow characteristics of powder. Both the mushroom powders gave comparable values of Carr's index and Hausner ratio. Extractive values indicate the type of biochemical constituents present in the sample. *Phellinus fastuosus* contained more alcohol soluble while *P. sanfordii* consisted of more water soluble polar biochemical components as indicated by the extractive values of each species. The absorption and emulsion properties form a criterion for the suitability of powder to be used in pharmaceutical and nutraceutical formulations. Each tested mushroom was observed showing comparable absorption and emulsion capabilities. The values of ash content of a powder give an estimation of inorganic or earthy material present in it. The value of ash content in each mushroom was found in the range recorded for sixteen wild mushrooms collected from Anatolia 3.37–15.46% [58]. The formation of foam and swelling of powder was not observed in any of the tested mushroom. The screening of mycochemical composition of each tested *Phellinus* species extract revealed the presence of various biochemicals and secondary metabolites such as carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenolic compounds, flavonoids, tannins, anthraquinone glycosides, cardiac glycosides and alkaloids. The results of mycochemical composition are in correlation with previous reports on macrofungi [59–62]. These mycochemical constituents are known to show various medicinal and physiological activities.

5. Conclusion

The results of the present findings provide beneficial information to identify these genuine species and can be employed to examine the purity and quality of these species in intact and pulverized form available commercially. The preliminary mycochemical screening of both *Phellinus fastuosus* and *P. sanfordii* wood inhabiting macrofungi is helpful in knowing the chemical composition of these mushrooms. The aggregate data obtained in this study can be utilized for the preparation of various pharmaceutical and nutraceutical products useful for human health. However, these mushrooms are grown in the wild and therefore for improved utilization, there is need to make them domesticate through culturing.

6. Acknowledgements

The authors are thankful to UGC (New Delhi) for providing financial support through MANF (Maulana Azad National Fellowship) awarded to the corresponding author during 2011–2016 and acknowledge Head, Department of Botany, Punjabi University, Patiala for providing the necessary laboratory facilities.

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