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RESEARCH ARTICLE

TAXONOMIC, PHYSICOCHEMICAL AND BIOCHEMICAL EVALUATION OF PHELLINUS ALLARDII (BRES.) S. AHMAD

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ABSTRACT

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Key words: Phellinus allardii, Taxonomy, Physicochemical, Biochemical. Phellinus Quél. (family Hymenochaetaceae) is a genus of wide occurrence and includes a number of species with great pharmacological significance. The present study is focused on taxonomic identification, physicochemical and biochemical evaluation of the specimen collected from district Dehradun, India. The specimen has been identified as Phellinus allardii on the basis of macroscopic and microscopic characters and is reported here as a new record for district Dehradun. Standardization using physicochemical parameters has been done following standard methods. The P. allarddii mushroom has high value of oil absorption capacity (740%), followed by water absorption capacity (486%), dispersibility (93%), dry weight (82.13%), emulsion capacity (27.38%), Carr's index (26.06%), emulsion stability (22.19%), moisture content (17.83%), total ash (4.16%), water soluble extractive (2.80%), alcohol soluble extractive (1.46%), acid insoluble ash (1.33%), Hausner ratio (1.32), water soluble ash (0.83%), tapped density (0.66 gmL⁻¹), bulk density (0.47 gmL⁻¹) and foreign matter (0.03%). The preliminary biochemical screening, according to the standard pharmacognostic procedures, has shown the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenols, flavonoids, tannins, anthraquinone glycosides and cardiac glycosides. The results of physicochemical parameters and biochemical analysis are the first to be reported for Phellinus allardii. The present investigation is indicative of the potential of this mushroom to be utilized as source of pharmaceutical and nutraceutical products.

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INTRODUCTION

There is an increasing demand worldwide of natural products for their use as medicines and nutraceuticals. Mushrooms are important resource for natural therapeutic agents and their demand is increasing day by day (Halpem, 2010; Dotan et al., 2011; De Sliva et al., 2012). Physicochemical and biochemical evaluation of various mushrooms Ganoderma lucidum, Agaricus sylvaticus; Lentinus subnudus, Phellinus linteus, Pleurotus ostreatus, Stereum ostrea and Lentinus edodes etc. (Enman et al., 2007; Fortes et al., 2009; Adedayo and Rachel, 2011; Isaka et al., 2011; Hseih et al., 2013; Singh et al., 2014; Usha and Suguna 2014) has been done for such use. Out of the various medicinal mushrooms studied so far species of genus Phellinus such as P. baumii, P. gilvus, P. igniarius and P. linteus etc. have been recognised as therapeutic agents for the management of various ailments (Anonymous, 1955; Kim et al., 2004; Hwang et al., 2005; Zhu et al., 2008; Lu et al., 2009; Kim et al., 2010).

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These effects are attributed to various bioactive constituents isolated from them (Kim et al., 2003; Bae et al., 2005; Wang et al., 2005; Lee and Yun, 2008; Park et al., 2009; Yang et al., 2009; Xue et al., 2010). However, no work on physicochemical and biochemical aspects has vet been conducted for *Phellinus allardii*. The present investigation taxonomic identification, pertains to evaluation of physicochemical parameters and preliminary biochemical screening of Phellinus allardii collected from district Dehradun, Uttarakhand, India with a view to establish standards for its identity, quality, purity and chemical composition. It is classified under family Hymenochaetaceae (order Hymenochaetales, class Agaricomycetes, sub-phylum, Agaricomycotina and phylum Basidiomycota). It is a cosmopolitan genus (Kirk et al., 2008). From India, it has earlier been reported and listed from different localities by various workers (Bakshi, 1971; Sharma, 1985; Singh, 1987; Sharma and Ghosh 1989; Sharma, 1995; Sharma, 1997; Leelavathy, 2000; Sharma, 2000; Forouton and Vaidya 2007; Forouton and Jaffary, 2007; Ranadive et al., 2011; Sharma, 2012; Prahser and Lalita, 2013; Kaur, 2013; Ranadive et al., 2013). However, it is given as a new record for district

Dehradun. It generally causes white rot in angiospermic trees. It is characterized by pileate, perennial basidiocarps which are generally some shade of brown with a black crust, stratified tubes, thin context in between the tube layers, 5-7 per mm, round to angular pores, with thick and entire dissepiments; dimitic hyphal system, ellipsoid to broadly ellipsoid, yellowish brown to golden brown, cyanophilous, inamyloid spores. Standardization of physicochemical parameters (Foreign matter, moisture content, dry weight, bulk density, tapped density, Carr's index, Hausner ratio, dispersibility, alcohol soluble extractives, water soluble extractives, oil absorption capacity, water absorption capacity, emulsifying activity, emulsion stability, total ash, acid insoluble ash, water soluble ash, foaming capacity, foaming stability and swelling index) has been done to identify, measure quality and purity levels of the sample available in powder form. The biochemical screening of ethanolic extract (70%) of the powder of the basidiocarp revealed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenols, flavonoids, tannins, and glycosides which may be responsible for its therapeutic potential in various medicines and food formulations.

MATERIAL AND METHODS

Collection of Fungi

The specimen was collected from Landour (Mussoorie, Dehradun, Uttarakhand, India) during the month of October in the year 2010. Field notes comprising habit of basidiocarp, host, name of locality and morphological characteristics of the collected specimen were recorded.

Taxonomic study

Macroscopic details of the basidiocarp pertaining to abhymenial and hymenial surfaces, context, and pore tubes have been observed using a hand lens. 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 (Ordynets, 2012).

Submission of the specimen

The description based on macroscopic and microscopic study has been compared with the published literature and the specimen has been identified as *Phellinus allardii*. The specimen has been assigned a herbarium number (PUN 6001) and submitted to the herbarium of the Department of Botany, Punjabi University, Patiala (Punjab).

Chemicals

The chemicals used for extraction and qualitative chemical screening are of AR grade (Himedia, Merck India and SD Fine Chemical Ltd., India).

Physicochemical evaluation

The powdered fruiting body of *Phellinus allardii* has been used for the determination of the following physicochemical parameters in triplicate.

Foreign matter

The powdered sample (650 g) was weighed and the pieces of foreign matter were sorted out by visual examination and use of a lens ($6\times$). All portions of foreign matter were pooled, weighed and percentage yield calculated (Indian Pharmacopoeia, 2007).

Moisture content

The powdered sample (2 g) was taken and dried in the oven at 105 °C until constant weight achieved. Loss on drying was calculated as percentage of loss of water on drying (Indian Pharmacopoeia, 2007).

Dry weight

This was taken as the final weight obtained after the sample have been dried in the oven at 105 °C for 24 hours until a constant weight was achieved (AOAC, 2000).

Extractive values

A known weight of 5 g powder was taken and maceration was done with100 mL of 90% ethanol in a 250 mL flask for 24 h after a frequent shaking for 6 h. This was followed by filtration and 25 mL of the filtrate was transferred to the already weighed china dish. Evaporation to dryness, cooling and then weighing was done. 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 (Indian Pharmacopoeia, 2007).

Ash values

About 2 g powder was ignited in a weighed crucible at temperature \leq 450 °C followed by cooling, weighing and then calculation of the percentage total ash was done. 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 a constant weight. Percentage of acid insoluble ash was calculated with reference to the air-dried powdered sample. For the determination of water soluble ash content 25 mL of chloroform water was taken in place of dilute HCl. The weight of the water insoluble ash subtracted from that of total ash provide the weight of the water soluble part of total ash. The percentage water soluble ash was calculated with reference to the initial mushroom powder sample taken (Indian Pharmacopoeia, 2007).

Absorption properties

One gram powder was mixed with 10 mL of distilled water or refined soybean oil. It was then kept standing undisturbed for 1 h at room temperature followed by centrifugation at 2000 rpm for 30 min. The supernatant thus obtained was taken in a 10-mL graduated cylinder. Water or oil absorption capacity was represented as volume of water or oil absorbed per gram of the dried powdered sample (Aremu *et al.*, 2007).

Emulsion values

In calibrated centrifuge tube 2g powder was taken followed by addition of 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 activity was calculated in percentage of the ratio of the height of the emulsified layer to the total height of the material in the tube. Emulsion stability was expressed as percentage of the total height of the material in the tube after heating the tubes at 80° C for half an hour. Then the contents were cooled for 15 min and centrifugation was done at 1600rpm for 15 min (Yatsumatsu *et al.*, 1972).

Dispersibility

Five gram of the mushroom powder was weighed and taken in a 100 mL measuring cylinder. Then distilled water was added to make the final volume to 100 mL. It was allowed to stand 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 (Kulkarni *et al.*, 1991).

Flow properties

A 50 g powder of the sample was weighed and put into a 100 mL measuring cylinder. The initial volume covered by the powder in the measuring cylinder was recorded and the bulk density was obtained as ratio of weight to volume V_B of sample. After this 500 manual taps were done and the volume occupied by the powder 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) = $V_B - V_T / V_B \times 100$

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 less than 15% indicates good flow characteristics and a value more than 25% indicates poor flow characteristics (Terangpi *et al.*, 2013).

Foaming properties

One gram mushroom powder was weighed and then 50 mL of 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 noticed and percentage foaming capacity was calculated. The amount of foam that remained stable after 30 min was taken as foaming stability (Aremu *et al.*, 2007).

Swelling index

To note the swelling property of 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 (Terangpi *et al.*, 2013).

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

Preparation of extract

The dried powder (150g) was extracted with 1500 ml of 70% ethanol (Harikrishnan *et al.*, 2010; Fig 1). The mixture was agitated for 72 h using orbital shaking incubator at 80rpm and 37^{0} C. The ethanol extract was filtered. The filtrate was then collected and the solvent was evaporated by simple distillation. The residue was then dried in a hot air oven at 45°C. The residue was weighed and percentage yield was calculated in terms of the air-dried weight of the mushroom and the organoleptic properties of the extract have been recorded as shown in (Table 3). The extract was stored at -4^oC for further use.

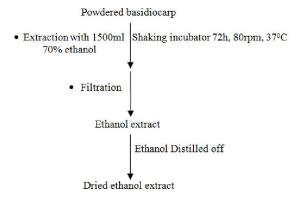


Figure 1. Preparation of extract

Qualitative chemical screening

The qualitative chemical examination of the extract was done following the standard methods (Kokate, 2004; Shah and Nayak, 2008; Trease and Evans, 2009).

RESULTS

Taxonomic studies

Phellinus allardii (Bres.) S. Ahmad, Basidiomycetes of West Pakistan: 57, 1972. Fig 2(a-f)

Morphology

Basidiocarps perennial, effused-reflexed to pileate, solitary to imbricate, boadly attached, triquetrous in section, woody hard, heavy, pilei $\leq 5 \times 8 \times 6$ cm; upper surface reddish brown to dark brown to black with age, concentrically sulcate with numerous narrow zones, crustose, crust ≤ 0.5 mm thick, rimose; lower surface glancing, greyish brown to brown when fresh, reddish brown to dark brown on drying; pores round to angular, 5–7 per mm; dissepiments vary in thickness from 33 to 133 µm, entire; pore tubes ≤ 2.5 mm long in each layer, greyish brown to brown, distinctly stratified; context between the tube layers very thin, ≤ 480 µm thick, brown; margins thinning, irregular, wavy, concolourous on the abhymenial side, light brown, sterile ≤ 1 mm on the hymenial side.

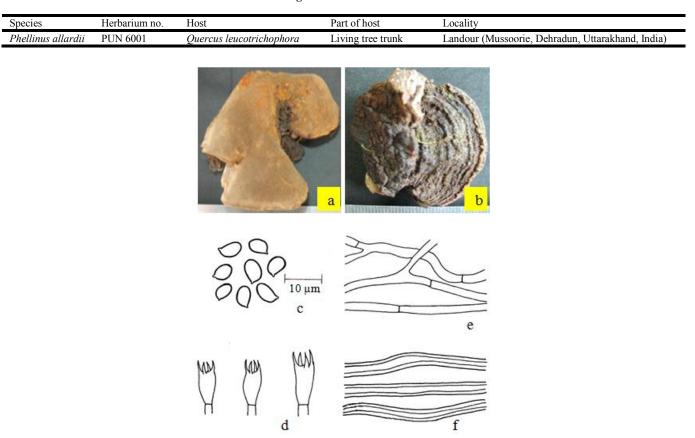


Table 1. Showing details of the collection examined

Figure 2. Showing the macroscopic and microscopic details of *Phellinus allardii*: a. Basidiocarp showing hymenial surface, b. Basidiocarp showing abhymenial surface, c. spores, d. basidia, e. generative hyphae, f. skeletal hyphae

Sr. No.	Parameter	n	
SI. NO.	Parameter	Mean ±SEM	
1.	Foreign matter (% w/w)	0.03±0.01	
2.	Moisture content (% w/w)	17.83±2.21	
3.	Dry weight (% w/w)	82.17±2.21	
4.	Extractive values (% w/w)		
	Ethanol (90%) soluble extractive	1.46±0.13	
	Water soluble extractive	2.80±0.46	
5.	Ash values (% w/w)		
	Total ash	4.16±1.16	
	Acid insoluble ash	1.33±0.33	
	Water soluble ash	0.83±0.16	
6	Absorption properties (%)		
	Water absorption	486±0.34	
	Oil absorption	740±0.15	
7.	Emulsion values (%)		
	Emulsion capacity	27.38±0.44	
	Emulsion stability	22.19±1.25	
8.	Dispersibility (% v/v)	93.00±0.57	
9.	Flow properties		
	Bulk density (g mL ⁻¹)	0.47 ± 0.02	
	Tapped density (g mL ⁻¹)	0.66±0.56	
	Hausner ratio	1.32±0.11	
	Carr's index (%)	26.06±3.94	
10.	Foaming properties (% v/w)		
	Foaming capacity	0.00	
	Foaming stability	0.00	
11.	Swelling index (% v/w)	00.00	
n=3			

Table 2. Physicochemical evaluation of Phellinus allardii

Microscopy of the specimen

Hyphal system dimitic. Generative hyphae $\leq 2.6 \ \mu m$ wide, branched, simple-septate, thin-to thick-walled, subhyaline to pale yellow.

Skeletal hyphae \leq 3.8 µm wide, unbranched, aseptate, thickwalled, yellowish brown to brown. Both generative as well as skeletal hyphae intertwined, horizontal in the context and vertical in the trama. Hymenial setae absent. Setal hyphae absent. Basidia $8.4-11.1 \times 3.9-5.2 \mu m$, clavate, subhyaline, 4-sterigmate; sterigmata $\leq 3.8 \mu m$ long. Basidiospores $4.5-6.5 \times 2.6-4.5 \mu m$, ellipsoid to broadly ellipsoid, smooth, thickwalled, yellowish brown to golden brown, darkening in KOH sol., cyanophilous, inamyloid. Details of the collection examined with reference to herbarium number, name of host, attachment to the host part are given in Table 1. visible, short UV (254) and Long UV (365) light inside the UV chamber. There was faint mushroomy odor of the extract and the consistency was sticky and semisolid. The biochemical evaluation of 70% ethanolic extract of the tested species as shown in Table 4 revealed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids,

Table 3. 1	Percentage yield and	organoleptic	properties of	f Phellinus	allardii extract

Color			Odor	Consistency	Yield of extract (% w/w DW
Visible light	Short UV (254 nm)	Long UV (365 nm)	-		Mean \pm SEM
Reddish brown to dark brown	Orange yellow	Bright yellow	Faint mushroomy	Sticky and semisolid	0.86 ± 0.01
n=3	0,		÷	<i>.</i>	
	r	Fable 4. Biochemic	al analysis of extract		
	Biochemical constitue	ent/ chemical test	Name of test	Inference	
	Carbohydrates		Molisch's test	++	
	Daduaing Sugara		Anthrone test	++ ++	
	Reducing Sugars		Fehling's test Benedict's test	++	
	Proteins		Xanthoproteic test	++ 	
	FIOLEIIIS		Lead acetate test		
			Millions test	++	
			Biuret test	++	
	Amino acids		Ninhydrin test		
	Annio acido		Lead acetate	++	
	Steroids		Hesse's test		
	Steroids		Mole Schott's test		
			Libermann's test		
			LB test	++	
	Terpenoids		Salkowski's test		
	responded		Liebermann Burchard's		
	Phenols		Folin-Ciocalteu test	++	
	Flavonoids		Shinoda test		
			Conc. Nitric acid tes	t ++	
			Alkaline reagent tes		
	Tannins		Bramer's test	++	
			Lead acetate test	++	
			Potassium dichromate	test ++	
	Anthraquinone glycos	ides	Borntrager's test		
	1 05		Modified Borntrager's	test ++	
	Cardiac glycosides		Baljet's test	++	
	0.7		Killer-Kiliani test	++	
	Cyanogenetic glycosic	des	Hydrogen cyanide te	st	
	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		
	e		Swelling test		

Physicochemical evaluation

The results of physicochemical properties of *P. allarddii* mushroom showed highest value of oil absorption capacity (740%), followed by water absorption capacity (486%), dispersibility (93%), dry weight (82.13%), emulsion capacity (27.88%), Carr's index (26.06%), emulsion stability (22.19%), moisture content (17.83%), total ash (4.16%), water soluble extractive (2.80%), alcohol soluble extractive (1.46%), acid insoluble ash (1.33%), Hausner ratio (1.32), water soluble ash (0.83%), tapped density (0.66 gmL⁻¹), bulk density (0.47 gmL⁻¹) and foreign matter (0.03%). The formation of foam and swelling of powder did not occur as shown in Table 2.

Organoleptic properties and biochemical screening

The organoleptic properties represented in Table 3 show variation in color of 70% ethanolic extract when exposed to

phenols, flavonoids, tannins, glycosides (anthraquinone and cardiac) and is lacking in cyanogenetic glycosides, alkaloids, lipids, saponins and mucilages.

DISCUSSION

Phellinus mushrooms belong to a diverse group of basidiomycete fungi that play an important role in the maintenance of human health as functional foods and in medicine since antiquity as they are found to be rich in various bioactive compounds (Tao *et al.*, 2005; Sun *et al.*, 2006; Zhu et al., 2008; Xue *et al.*, 2010). The diagnostic morphological and microscopic characters of the specimen helped to identify it as *Phellinus allardii* and this is a new record for the study area. Standardization using physicochemical parameters has been done following standard methods. The physicochemical analysis of *Phellinus allardii* for foreign matter, moisture

content, dry weight, ash content and extractive values help in determining the quality and purity of the mushroom sample (WHO, 1998). The presence of higher amounts of foreign matter in a drug or food formulation may pose adverse impacts on health. Therefore, this parameter should not be neglected. As per standards the acceptable upper limits for foreign matter should be $\leq 2\%$ (Soni *et al.*, 2011). Our results showed much low foreign matter content. High moisture content hikes susceptibility to microbial attack due to the elevation of enzyme activities which promote spoilage. The percentage moisture content 17.83% was found less compared to the values reported previously for *Lentinus subnudus* (78%), Chlorophyllum molybditis (94%), Marasmus species (84%) and Pleurotus tuber-regium (89%), Phellinus linteus and Phellinus wahlbergii (36.6-48.4%) respectively (Adedayo and Rachel 2011; Meghlatha et al., 2014). Dry matter (82.17%) was found higher 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 (Nwanze et al., 2006; Usha and Suguna, 2014). Ash values of a drug provide an idea about earthy matter or inorganic composition and other impurities present in the drug. Extractive values indicate the nature of chemical constituents present in the drug.

Water soluble extractive 2.80% was found higher as compared to that for alcohol soluble extractive 1.46% showing that it had less alcohol soluble polar constituents that was further supported by low percentage yield of extract 0.86%. The ash content in the present study 4.16% was reported above the values (0.85–0.90%) found in Lentinus subnudus, Chlorophyllum molybditis, Marasmus species and Pleurotus tuber-regium, 1.3% for Phellinus linteus and below the values (5.56-6.30%) known for Phellinus wahlbergii and Phellinus linteus respectively (Adedayo and Rachel 2011; Meghlatha et al., 2014 and Reis et al., 2014). The acid insoluble ash is an indicative of silicate impurities while water soluble ash represents the highly soluble mineral content in the sample. The values for acid insoluble ash 1.33% and water soluble ash 0.83% in the investigated mushroom were found much less than for acid insoluble ash and 18% for water soluble ash respectively reported in Ganoderma sinense (Chittaragi and Naika, 2014). Absorption properties and emulsion properties were also found favorable, making Phellinus powder available for use in many drug formulations where emulsification and reconstitutability are required. The ability of association of powder and water or oil is provided by its absorption properties which gives an information about the incorporation of powder or isolates whether they are suitable for their incorporation into aqueous or oily nutraceutical and pharmaceutical formulations (Udensi and Okoronkwo, 2006). The oil absorption capacity 740% and water absorption capacity 486% was found higher than 30.2% and 337% as reported for Pleurotus tuberregium flour and protein concentrate (Arubi and Alobo, 2003). The ability of powder to emulsify oil is described by its emulsion properties which play an important role in drug preparations, cosmetics, pastes or cod liver oil. Emulsions have also been used for ameliorating dermatological disorders, lacerations and as drug delivery agents etc. (Khan et al., 2011). Emulsion capacity 27.38% was less than 30.0% and emulsion stability 22.19% of Phellinus allardii was greater than that of Pleurotus tuberregium sclerotia flour but lower than known for its protein concentrate (Arubi and Alobo, 2003). Bulk density is a criterion to measure the heaviness of powder sample which provides the relative volume of the packaging material needed and dispersibility of powder in water gives an idea of its reconstitutability (Kulkarni et al., 1991). The value of bulk density was 0.47 g/mL which is higher than 0.37-0.40 g/mL and lies within the range 0.45-0.49 g/mL) known for Pleurotus tuberregium sclerotia flour and protein concentrate and Ganoderma lucidum and Ganoderma philippii respectively (Singh et al., 2014; Arubi and Alobo, 2003). The tapped density, Flow properties indicate that powder may be utilized as a direct compression excipient. Hausner ratio provides interparticle friction and Carr's index is a measurement of compressibility of powder. Hausner ratio and Carr's index of Phellinus allardii was observed higher than 1.25 and 15% respectively.

The dispersibility value was found to be 93% which is higher than (53.97-59.6%) noted for Ganoderma lucidum and Ganoderma philippii respectively (Singh et al., 2014). The ability of mushroom powder to form foam is determined by its foaming properties. In the present study, no foam formation had been reported which can be attributed to the absence of saponins. Saponins are known to contribute in the process of foam formation (Chen et al., 2010). Swelling index indicates that no swelling of powder had been observed as Phellinus allardii was lacking mucilage substances. The organoleptic properties provide important information which may be helpful in authentication and detection of adulteration for quality control of raw material. The results of biochemical analysis of Phellinus allardii showed the presence of carbohydrates, reducing sugars, proteins, amino acids, steroids, terpenoids, phenols, flavonoids, tannins and glycosides (anthraquinone and cardiac). The results of qualitative chemical screening are in correlation with previous reports on mushrooms (Kim et al., 2003; Maiti et al., 2008; Park et al., 2009; Jang et al., 2010; Soni et al., 2011; Akata et al., 2012; Kulkarni, 2013; Hseih et al., 2013; Meghlatha et al., 2014; Reis et al., 2014; Toopmuang et al., 2014).

Conclusion

The present investigation provides useful information which helps in identification of *Phellinus allardii* and establishing standards to check for adulteration of intact fruit bodies and powder available commercially. The preliminary biochemical results are helpful in finding the chemical constituents that may have medicinal and nutraceutical applications and may prove beneficial for human health.

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