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A Study Of Taxonomy, Physicochemical Features And Chemical Constituents Of A Few Edible Mushroom Species.

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ABSTRACT

Pleurotus ostreatus, *Calocybe indica* and *Volvariella volvacea* are commonly used edible mushrooms that are valued for their medicinal and nutraceutical properties since ancient times. The present study aims to provide quality control standards for selected edible mushrooms by evaluating their taxonomy, physicochemical parameters and chemical constituents. The taxonomic studies showed highlighted the macroscopic and microscopic characters including external basidiocarp characteristics and shape and size of basidiospores. The fresh basidiocarps were air dried, powdered and subjected to physicochemical analysis. Aqueous extracts of the mushrooms were prepared by refluxing powder with distilled water and then subjected to qualitative and quantitative chemical screening. The results showed variation among physiochemical parameters like foreign matter, moisture content, ash content, extractive values, absorption properties, emulsion properties, foaming properties, dispersibility, and bulk density. Qualitative chemical screening of aqueous extracts revealed the presence of carbohydrates, flavonoids, tannins, phenolic compounds, sterols and proteins in all three mushrooms. Quantification of chemical groups showed significant amount of phenols, flavonoids and carbohydrates indicating medicinal potential of *P. ostreatus*, *C. indica* and *V. volvacea*. These standardisation profiles of the chosen mushrooms will help in authentication, analysis of purity content and chemical composition of these mushrooms.

Keywords: *Pleurotus ostreatus*; *Calocybe indica*; *Volvariella volvacea*; basidiocarp; quality standards

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INTRODUCTION

Mushrooms have been the centre of attraction as food, medicine and cosmetics throughout the world [1,2]. From approximately 0.14 million mushroom species only 14,000 have been identified of which 7,000 are considered as safe for consumption and 700 are recognised to possess pharmacological properties [3,4]. Since ancient times, mushrooms have been considered as a delicacy for their desirable taste as well as their high nutritional and functional value [5]. They are extensively used in folk medicine and have also been accepted as nutraceuticals offering health benefits and protection from degenerative diseases [6.] Edible mushrooms are also a valuable source of biologically active compounds. The genus, *Pleurotus*, comprises about 40 species and is grown widely in temperate and tropical areas [7]. Among them *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm (Pleurotaceae) (known as oyster mushroom) is an edible mushroom, having excellent taste and has been used traditionally to treat headaches, stomach aches and asthma [8-11]. Interest has increased considerably in this species because of its gastronomic value and nutraceutical properties. Many medicinal properties including antibacterial, anticancer, antioxidant has been explored extensively [12-14]. *Calocybe indica* Purkayastha & A. Chandra (Lyophyllaceae), also known as milky mushroom, is a tropical edible mushroom of Indian origin and cultivated in high temperature and humidity conditions[15]. It has considerable nutritional value and has been extensively explored for its medicinal properties like antidiabetic, anti-tumour and anti-inflammatory [16,17]. *Volvariella volvacea* (Bull. Ex Fr.) Sing. (Pluteaceae) is an edible mushroom widely cultivated in China and other regions of Southeast Asia [18]. It is also known as paddy straw mushroom and contains a number of active principles like polysaccharides, steroids, lectins, proteins, volatile components which are anti-oxidant, anti-bacterial, anti-fungal and anti-tumour [7,19-24]. Thus these edible mushrooms need special consideration due to their high nutritional and medicinal values.

In spite of tremendous medicinal potential, the quality control standards are not available for *Pleurotus* ostreatus, *Calocybe indica* and *Volvariella volvacea*. Thus, in the present investigation, a comparative evaluation of the taxonomic characters, physiochemical parameters and chemical constituents of fruiting bodies of *P. ostreatus*, *C. indica* and *V. volvacea*, was performed with a view to determine their identity, quality, purity, and chemical composition.

MATERIALS AND METHODS

Collection of fungi

The fruiting bodies of edible mushrooms (*Pleurotus ostreatus*, *Calocybe indica* and *Volvariella volvacea*) were obtained in month of September, 2014 from Directorate of Mushroom Research Solan, Himachal Pradesh, India (receipt no. 11024 and 11086) which is located at a height 1500 m above sea level. The mushrooms were cultivated on the remnants of poplar tree (*Populus* sp.).

Chemicals

Ascorbic acid, dextrose, methanol, gallic acid and petroleum ether were procured from Loba Chemical Ltd., India, while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich, USA. All the other chemicals and reagents used were of analytical grade.

Taxonomic studies

The basidiocarps were evaluated by studying their shape, size, colour, texture, hymenial surface, gills, context, type of gills and margin. The colour standards used are as per Methuen's Handbook of Colors [25]. Microscopic details related to hyphae, basidia and basidiospores of the specimens and their arrangements were studied by making crush mounts and hand cut sections respectively in water, 3% KOH solution and staining them with Congo red, Phloxine, cotton blue and melzer's reagent [26].

Physicochemical evaluation

The physicochemical parameters for *P. ostreatus*, *C. indica* and *V. volvacea* were evaluated and determined in triplicate.

Ash content

Total ash was determined by incinerating air dried powdered sample (2-3 g) at a temperature not exceeding 450 °C until carbon free. It was cooled, weighed and percentage total ash was calculated. For acid insoluble ash, total ash was boiled with 25 ml of 2 M HCl for 5 min, filtered through ash less filter paper, washed with hot water, and ignited at a temperature not exceeding 450°C until constant weight. The percentage yield was calculated with respect to the air dried powdered sample. For water soluble ash, chloroform water (25 ml) was taken instead of HCl[27].

Foreign matter

Foreign matter from the air dried powdered sample (100 g) was collected by visual inspection and use of a lens (6×). Foreign matter was weighed, and percentage yield was calculated with respect to the air dried powdered sample [27].

Moisture content

The fresh mushrooms were cut into small pieces, weighed accurately (2 g) and dried in an oven at a temperature not exceeding 105°C until constant weight. The loss on drying was determined by the difference in the weight of fungal material before and after drying and calculated as percentage loss of moisture on drying [27].

Extractive values

Accurately weighed (4 g) powdered fungal material and macerated with 100 ml water or hydro-methanol solution (80:20) in closed flasks, with frequent shaking for 6 h and then allowed to stand for 18 h. The percentage extractive value was calculated on dry weight basis. For hot water soluble extractive, powdered fungal material (4 g) was weighed and refluxed with 100 ml of water for 2 h. The solution was filtered and 25 ml of the filtrate was evaporated to dryness, cooled and weighed. The percentage hot water soluble extractive was calculated with respect to the air dried fungal material [27].

Absorption properties

Accurately weighed air dried powdered sample (1 g) was mixed with 10 ml of distilled water or refined soybean oil. The mixture was allowed to stand at room temperature for 30 min. It was then centrifuged and the volume of supernatant was noted. Water and oil absorption capacity was expressed as volume of water or oil absorbed per gram of the air dried powdered sample.

Emulsion properties

Accurately weighed air dried powdered sample (1 g) was blended with distilled water (50 ml) for 30 s followed by addition of refined soybean oil in increments of 5 ml until separation into 2 layers appeared. The obtained emulsion was allowed to stand at room temperature. The volume of emulsion formed per gram of powdered sample was calculated. The volume of emulsion which remained stable for 30 min was recorded as emulsion stability [28].

Foaming properties

Accurately weighed air dried powdered sample (1 g) was dispersed in distilled water (50 ml), whipped vigorously for 30 min and poured into a 100 ml graduated cylinder. The volume before and after whipping was recorded and foaming index was calculated in percentage. Foaming stability was the amount of foam that remained stable after 30 min [28].

Dispersibility

Accurately weighed air dried powdered sample (5 g) was added to a measuring cylinder, and volume was adjusted to 100 ml with distilled water. The mixture was stirred and allowed to stand for 1 h. The volume

of settled particles was noted and subtracted from 100 and difference was reported as percentage dispersibility [29].

Bulk density

Accurately weighed air dried powdered fungal material (50 g) was added to a 100 ml measuring cylinder and tapped to constant volume. The bulk density was calculated as weight per unit volume of sample [29].

Preparation of extracts

Fungal material was air dried in shade and reduced to coarse powder. The powdered sample was defatted using petroleum ether 60-80°C. The defatted material was extracted with hot water as described in Figure 1. The extract was concentrated under vacuum. The percentage yield and organoleptic characters for each extract were noted.

Qualitative chemical screening

The qualitative chemical screening of extracts was done by following standard procedures [30,31].

Quantification of chemical groups

Total phenol content (TPC) analysis

Total phenolic content of extracts was determined by Folin-Ciocalteu procedure [32,33]. The amount of total phenols was calculated as gallic acid equivalent from the calibration curve of standard gallic acid and expressed as milligram gallic acid equivalent (mg GAE) per gram of extract. All measurements were done in triplicate.

Total flavonoid content (TFC) analysis

Total flavonoid content of extracts was determined by aluminium chloride method [34]. The flavonoid content was determined as quercetin equivalent from the calibration curve of quercetin standard solutions and expressed as milligram quercetin equivalent (mg QE) per gram of extract. All measurements were done in triplicate.

Total carbohydrate content (TCC) analysis

The total carbohydrate content was estimated using anthrone method [35]. The anthrone reaction is used for the determination of hexoses, aldopentoses and hexauronic acids, either free or present in polysaccharides. The total carbohydrate content was calculated as glucose equivalent from the calibration curve of glucose standard solution and expressed as milligram glucose equivalent (mg GE) per gram of extract. All measurements were done in triplicate.

RESULTS

Taxonomic studies

The basidiocarps of *Pleurotus ostreatus*, *Calocybe indica* and *Volvariella volvacea* were examined on the basis of both external features as well as microscopic structures, compiled into description, which were compared with literature [36-41]. Taxonomic features of these fungi are given in Table 1.

Pleurotus ostreatus (Jacq.) P. Kumm., Der Führer in die Pilzkunde: 105, 1871.

Syn.: *Agaricus ostreatus* Jacq., Flora Austriaca: 104, 1774.

It is macroscopically characterised by clustered basidiocarps, with a funnel-shaped pileus, decurrent gills on the abhymenial surface and a lateral stipe, and microscopically by the presence monomitic hyphal system, non-inflated generative hyphae with clamp connections, and cylindrical basidiospores (Figure 2).

Calocybe indica Purkayastha & A. Chandra, Transactions of the British Mycological Society 62(2): 415, 1974.

It is macroscopically characterised by caespitose basidiocarps of varying sizes, with a convex to plano-convex pileus, crowded sinuate gills on the abhymenial surface and a central stipe with a bulbous base, and microscopically by the presence of monomitic hyphal system, thin-walled generative hyphae with clamp connections, and broadly ellipsoid basidiospores (Figure 3).

Volvariella volvacea (Bull.) Singer, Lilloa 22: 401, 1951.

Syn.: *Agaricus volvaceus* Bull., Herbier de la France 6: 262, 1786.

It is macroscopically characterised by gregarious basidiocarps, with umbonate pileus, and a centrally placed stipe covered with a sac-like volva at the base, and microscopically by monomitic hyphal system and oval to ovoid thin-walled basidiospores (Figure 4).

Physicochemical evaluation

The physicochemical parameters for powdered samples are shown in Table 2. Our results show higher water soluble extractive values than corresponding alcohol soluble extractive values for all the mushrooms. *C. indica* has a higher ash value as well as higher water absorption capacity while higher values of foaming and emulsion properties were recorded for *P. ostreatus*.

Preparation of extracts

The percentage yields along with organoleptic characters of the prepared extracts are reported in Table 3. The qualitative phytochemical screening revealed the presence of carbohydrates (non-reducing sugars), phenolic compounds, flavonoids, saponins and amino acids in all the prepared extracts while alkaloids, reducing sugars and glycosides were detected in none of the prepared extracts (Table 4).

Quantification of chemical groups

Total phenol, total flavonoid and total carbohydrate contents were quantified in all three mushrooms extracts and results are given in Table 5. Our results show high amounts of carbohydrates and phenolic compounds in all extracts. High values of phenol and flavonoids were recorded for *C. indica* extract in comparison to other fungal extracts.

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Table 1- Comparison of taxonomic features of *Pleurotus ostreatus*, *Calocybe indica* and *Volvariella volvacea*

Characters		*Pleurotus ostreatus*	*Calocybe indica*	*Volvariella volvacea*
Basidiocarp				
	Nature	Clusters	Caespitose	Gregarious
	Size	1.0-1.5 cm	8-15 cm	6-8 cm
	Colour	Young fruiting body pinkish tinge	Young fruiting body whitish	Young fruiting body greyish white
Pileus				
	Diameter	6-10 cm	5-18 cm	4-5 cm
	Colour	Pinkish tinge	Whitish	Overall white to brownish at centre, reddish brown towards margins on maturity
	Shape	Fan-shaped to funnel shaped	Convex to plano-convex	Campanulate
	Apex	Plane to depressed apex	Plane apex	Later broad umbonate
	Peel	Easily peeled	Easily peeled	Easily peeled
Hymenial surface		Moist	Moist	Moist
Margin		Enrolled when young, wavy or lobed at maturity	Regular	Irregular, splitting at maturity
Gills				
	Nature	Decurrent, unequal, close	Sinuate, unequal, crowded, dentate	Free, crowded
	Colour	Whitish with tinge of pink, yellowish on drying	Whitish	White to pinkish
Stipe				
	Placement	Lateral	Central	Central
	Size	0.5-1.0 × 0.5-1.0 cm	4-15 × 1-2 cm	5.5-7.0 × 0.5-1.0 cm
	Colour	Pinkish tinge	Whitish	White
	Base	Solid	Cylindrical with sub-bulbous base, solid	Tapering, solid
Surface		Silky	Fibrillose	Silky
Volva		Absent	Absent	Sac-like, brownish-grey
Hyphal system				
	Nature	Monomitic	Monomitic	Monomitic

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	Hyphae	Non-inflated generative hyphae	Generative hyphae	Highly branched generative hyphae
	Size	up to 5.5 µm wide, thin-walled	up to 7.3 µm wide, thin-walled	up to 5.5 µm wide, thin- to thick-walled
	Septa	Present	Present	Present
	Clamp connection	Present	Present	Absent
Basidiospores				
	Nature	Smooth, hyaline, inamyloid	Smooth, thin-walled, prominent apiculus, hyaline, non-amyloid	Smooth, thin-walled
	Size	6.7-9.7 × 3.6-5.5 µm	8.5-9.1 × 6.1-7.9 µm	9.7-10.9 × 6.7-8.5 µm
	Shape	Cylindric	Broadly ellipsoid	Oval to ovoid
Basidia				
	Shape	Clavate to clavate cylindric	Clavate	Clavate
	Size	26-28 × 6.7-7.3 µm	29-31 × 10.9-11.5 µm	27.9-30.3 × 10. 3-10.9 µm
	Sterigmata	Four	Four	Four

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Table 2- Physicochemical parameters of dried mushrooms

Physicochemical parameters	*P. ostreatus* (Mean^n ± SD)	*C. indica* (Mean^n ± SD)	*V. volvacea* (Mean^n ± SD)
Ash values (% w/w)			
Total ash	6.95 ± 0.14	7.69 ± 0.08	6.85 ± 0.12
Acid insoluble ash	3.58 ± 0.08	4.22 ± 0.02	3.56 ± 0.04
Water soluble ash	1.98 ± 0.01	1.25 ± 0.08	1.13 ± 0.02
Foreign organic matter (% w/w)	0.32 ± 0.01	0.12 ± 0.01	0.27 ± 0.01
Loss on drying (% w/w)	89.5 ± 0.01	91.99 ± 0.01	73.52 ± 0.01
Cold soluble extractive values (% w/w)			
Water soluble extractives	12.28 ± 0.01	10.89 ± 0.02	14.87 ± 0.01
Alcohol soluble extractives (Methanol)	4.29 ± 0.02	7.86 ± 0.01	7.66 ± 0.01
Hot soluble extractive values (% w/w)			
Water soluble extractives	20.85 ± 0.13	21.49 ± 0.11	24.69 ± 0.08
Absorption properties (ml/g)			
Water absorption capacity	4.85 ± 0.14	6.85±0.11	3.98 ± 0.13
Oil absorption capacity	8.36 ± 0.11	7.15 ± 0.10	6.58 ± 0.09
Emulsion property (ml/g)			
Emulsion capacity	12.89 ± 0.30	12.15 ± 0.28	10.98 ± 0.42
Emulsion stability	6.87 ± 0.12	5.66 ± 0.24	4.87 ± 0.11
Foaming property (% v/v)			
Foaming capacity	42.81 ± 0.58	23.64 ± 0.47	35.29 ± 0.17
Foaming stability	25.96 ± 0.22	16.85 ± 0.23	29.78 ± 0.11
Dispersibility (% w/v)	40.88 ± 0.25	53.22 ± 0.35	39.98 ± 0.25
Bulk density (mg/ml)	0.98 ± 0.01	0.87 ± 0.01	1.85 ± 0.01

(n=3)

Table 3- Percentage yield of hot water extracts of *P. ostreatus*, *C. indica* and *V. volvacea*

Mushroom	Extract	Colour	Consistency	Yield of extracts (% w/w)
P. ostreatus	Hot water	Dark brown	Sticky	18.97
C. indica	Hot water	Dark brown	Sticky	21.82
V. volvacea	Hot water	Dark brown	Sticky	25.15

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Table 4- Results of phytochemical screening

Class of compounds	Name of test	POE	CIE	VVE
Alkaloids	Dragendorff's test	-	-	-
	Mayer's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Carbohydrates	Molisch's test	+	+	+
	Fehling's test	-	-	-
	Benedict's test	-	-	-
Anthraquinone glycosides	Borntrager's test	-	-	-
Cardiac glycosides	Legal's test	-	-	-
	Baljet test	-	-	-
	Keller killiani test	-	-	-
C-glycosides	Modified borntrager's test	-	-	-
Flavonoids	Shinoda test	+	+	+
	Alkaline reagent test	+	+	+
	Concentrated H_2SO_4 test	+	+	+
Tannins and phenolic compounds	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+
Sterols	Salkowski's reaction	-	-	-
	Liebermann burchard test	-	-	-
Saponins	Froth test	+	+	+
Proteins	Biuret's test	+	+	+
	Millon's test	+	+	+
Amino acids	Ninhydrin test	+	+	+

+: present; -: absent; POE: *P. ostreatus* extract; CIE: *C. indica* extract: VVE, *V. volvacea* extract.

Table 5- Estimation of TPC, TFC and TCC

Mushroom extract	TPC (mg GAE/g) (Mean^n ± SD)	TFC (mg QE/g) (Mean^n ± SD)	TCC (mg GE/g) (Mean^n ± SD)
P. ostreatus	18.66 ± 0.23	2.22 ± 0.06	2854.82 ± 254.23
C. indica	29.04 ± 0.12	2.67 ± 0.11	1424.14 ± 142.82
V. volvacea	10.74 ± 0.28	1.43 ± 0.12	2046.10 ± 102.93

(n=3)

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Figure 1. Preparation of extracts

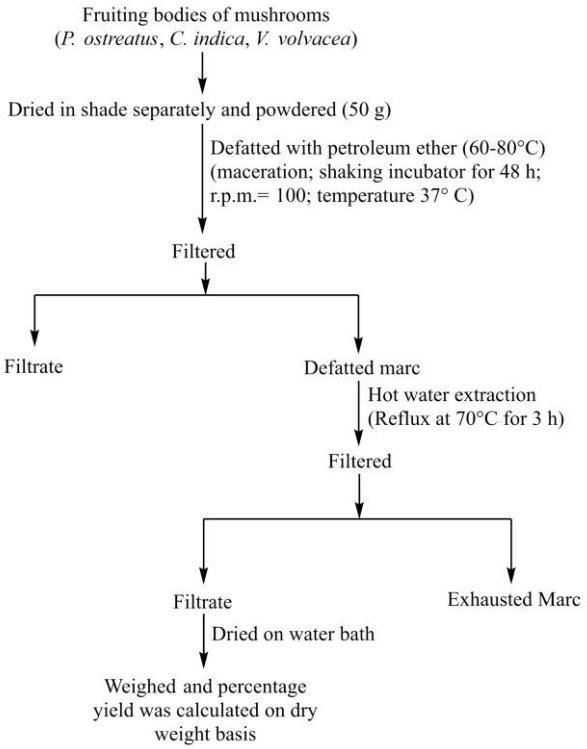

Figure 2. *Pleurotus ostreatus* (Jacq.) P. Kumm: a- basidiocarp, b- dried basidiocarp, c- basidiocarp showing hymenial and abhymenial surfaces, d- basidiospores, e- generative hyphae, f- basidia

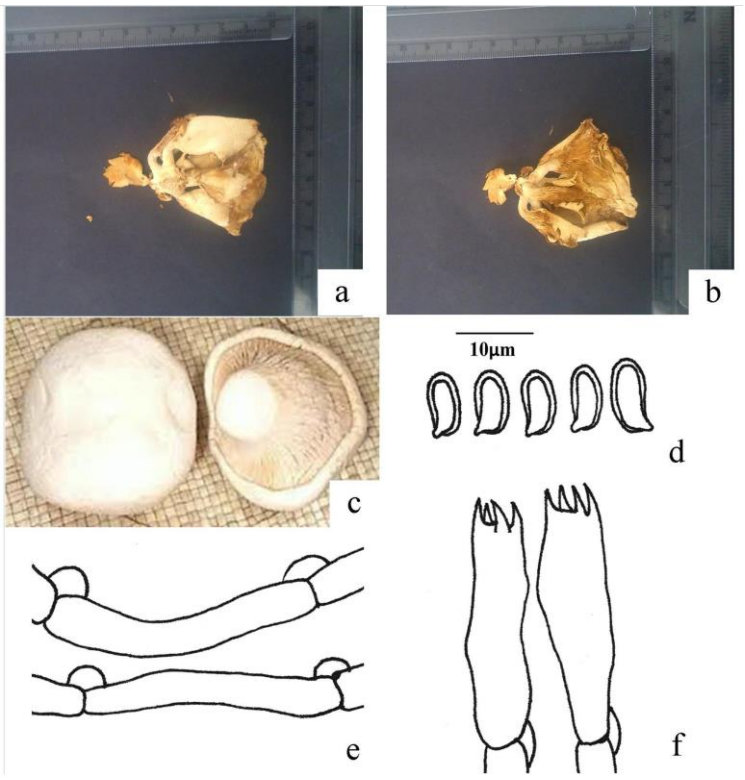

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Figure 3. ***Calocybe indica***: **a- basidiocarp showing hymenial surface, b- basidiocarp showing abhymenial surface, c- dried basidiocarp, d- basidiospores, e- generative hyphae, f- basidia**

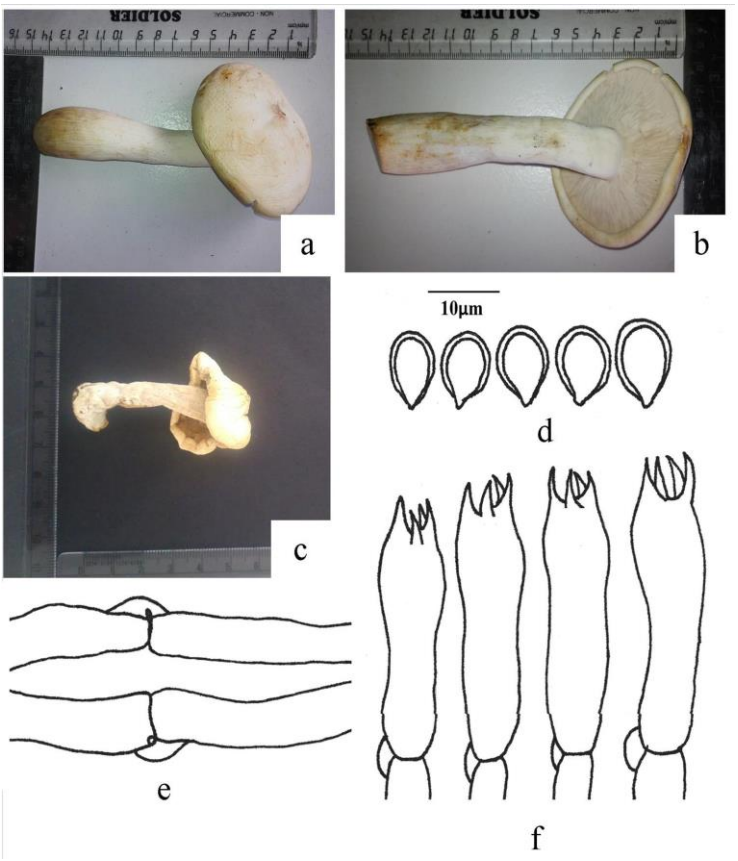
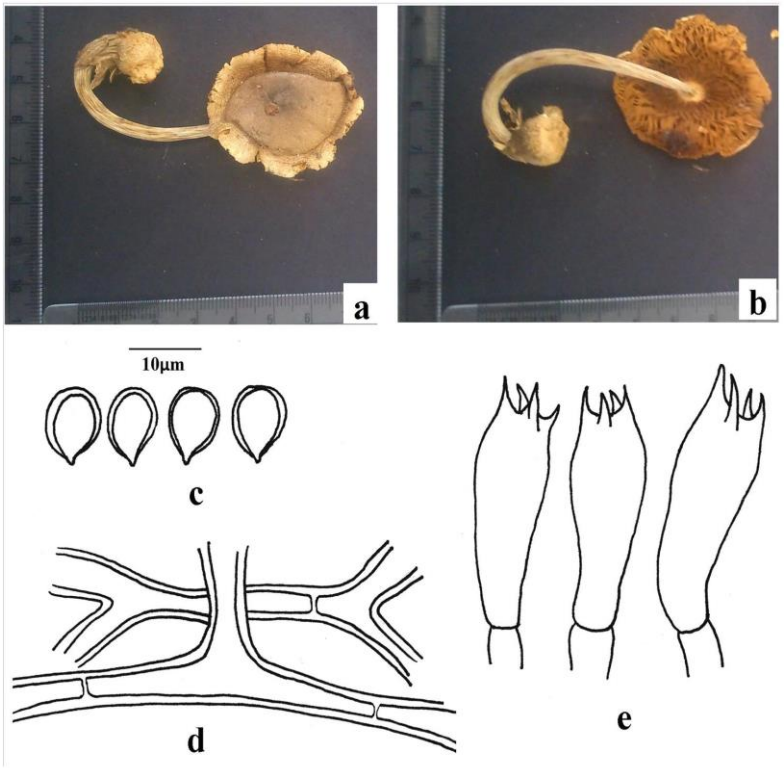


Figure 4. ***Volvariella volvacea***: **a- basidiocarp showing hymenial surface, b- basidiocarp showing abhymenial surface, c- basidiospores, d- generative hyphae, e- basidia**



DISCUSSION

Macroscopic and microscopic evaluation is the preliminary step for establishing the quality control profile of any herbal drug. These are the simplest and very reliable means to establish the correct identity of the source materials [41]. Macroscopic evaluation was employed to detect the presence of any foreign material as well as for identification of the class of the mushroom. The basidiocarps were studied in detail with respect to various features like shape, size, colour, type of gills and margin. The results were in conjunction with previous reports [7,42]. Microscopic evaluation was done to identify the mushroom species. Microscopic examination included assessment of hyphal system (type, size, wall thickness, septation), basidia (size, type, sterigmata) and basidiospores (size, shape, surface, apiculus). The identification markers were found to be similar to the previously reported features [40].

In order to check adulteration, quality and purity of the material, physico-chemical parameters like foreign matter, moisture content, ash content and extractive values are used [27]. Moisture content of the fresh basidioacarps analysed ranged from 73.52 to 91.99 % w/w. High moisture content promotes susceptibility to microbial growth and enzyme activity, which may further have critical impact on health of the consumer. The percentage moisture content values of *P. ostreatus* and *V. volvacea* (89.5 ± 0.01 and 73.52 ± 0.01 % w/w) were lesser than reported previously [43], while of *C. indica* (91.99 % w/w) was comparable to previous reports [44]. Ash content of a drug indicates presence of various impurities like carbonates, oxalates and silicates present along with the drug. The water soluble ash is used to determine the amount of inorganic compounds present in herbal drugs. Acid insoluble ash gives an idea about the amount of silica present and indicates contamination with earthy material. The results of this study showed that the ash values were comparatively higher than the previously reported [44]. Extractive values are useful to evaluate the amount and nature of specific constituents soluble in a particular solvent. Water soluble extractives were higher as compared to alcohol soluble extractives, which showed that *P. ostreatus*, *C. indica* and *V. volvacea* had more water soluble polar constituents. Our results also suggest that at high temperature better yield of extract could be obtained which might be due to decreased solvent viscosity, better penetration of matrix particles, enhanced diffusion rate and analytes' solubility at high temperatures [45].

For efficient utilization and consumer acceptance of mushroom flours, it is desirable to study their absorption, emulsion, foaming properties as well as their dispersibility and bulk density. Quality attributes of developed food and drug products are generally affected by these functional properties of the powder. These properties play a significant role in handling of powder material, retention of its flavour, improvement of palatability, reconstitutability and extension of shelf life of the pharmaceutical preparation of the powder [29]. These parameters have been evaluated for the first time for these mushrooms and may be employed in future to determine the quality of the species.

Extraction methods are generally used so that the known and unknown compounds from the material can be extracted in a high yield as well as in high purity [46]. The selected mushrooms were first defatted using petroleum ether 60-80°C by macerating for 48 h and then hot water extract of the defatted material was prepared using reflux at 70°C for 3 h. Since, higher extractive values with hot water (reflux condensation) were obtained thus, this method was chosen for extract preparation. The % yield obtained for *P. ostreatus*, *C. indica* and *V. volvacea* was 18.97 % w/w, 21.82 % w/w and 25.15 % w/w, respectively. Our results for qualitative chemical screening are parallel with previous reports on these mushrooms [47,48] and indicate the high nutritional and medicinal values of *P. ostreatus*, *C. indica* and *V. volvacea*.

On the basis of qualitative chemical screening results, the prepared extracts were standardised with respect to total carbohydrates, total phenols and total flavonoid content. Phenols and flavonoids are secondary metabolites having antioxidant, anticancer, antidiabetic, anti-inflammatory, hepatoprotective, neuroprotective properties [49-51]. Significant amounts of phenol, flavonoids and carbohydrates were found in *P. ostreatus*, *C. indica* and *V. volvacea* indicating the beneficial potential in various chronic diseases.

CONCLUSIONS

The present study provides quality control standards that may help to check adulteration of intact fruit bodies and commercially available mushroom powder of genuine species of *P. ostreatus*, *C. indica* and *V. volvacea*. The results obtained from the qualitative screening of the mushrooms' powder may provide basis for

the evaluation of processed flour either as food or as a nutraceutical, and the chemical tests are helpful in determining the chemical constituents that may have therapeutic properties for various pharmacological events. Thus, *P. ostreatus*, *C. indica* and *V. volvacea* mushrooms could be developed as candidates for drug development.

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