

## Qualitative and quantitative evaluation of nutritional and nutraceutical components of *Pleurotus cystidiosus* O.K. Miller

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### ABSTRACT

The genus *Pleurotus* (Fr.) P. Kumm. is a Pleurotoid mushroom having high nutritional and therapeutic value. In this manuscript the results of the investigations undertaken to evaluate the nutritional and nutraceutical components of local strain of *Pleurotus cystidiosus* on qualitative and quantitative basis has been presented. Upon evaluation the mushroom sample showed the presence of 9.61% ash content, 3.08% moisture content, 4.2% proteins, 79.45% carbohydrates, 1% crude fats, 2.66% crude fibers, 343.62 kcal/100g energy value, 0.97% phenols, 1.11% flavonoids, 1.02 µg/g β-carotene, 0.44 µg/g lycopene, 0.93% alkaloids and 0.14% steroids on dry weight basis.

**Key words:** Oyster mushroom, methanolic extract, bioactive compounds, nutritional and nutraceutical importance

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There is a common saying “medicines and foods have a common origin”, (Chang and Miles, 2008). Mushrooms are the manifestation of this idea because they are considered as a source of delicacy with high nutritional value and a potent source of nutraceuticals (Chang and Miles, 2008; Ergonul *et al.*, 2013). The term “functional foods” can be used for mushrooms since their dietary constituents have health benefits because of their cardio protective and antioxidant properties, which are beyond basic nutrition (Ferreira *et al.*, 2010). Oyster mushrooms represented by the genus *Pleurotus* occupied the second position among the globally cultivated mushroom species (Banik and Nandi, 2004) and is also considered as potent functional food items by various investigators (Synytsya *et al.*, 2008; Patel *et al.*, 2012) because of their significant positive effects on human health. The

oyster mushrooms are reported to contain various bioactive compounds, *i.e.* alkaloids, phenols, flavonoids, terpenoids, lectins and nucleotides with promising biological effects (Lindequist *et al.*, 2005). A number of mycochemicals with therapeutic relevance are present by edible *Pleurotus* species (Krishnamoorthy and Sankaran, 2014). Further these mushrooms possess the capability to recycle certain agricultural and industrial waste and can be produced in short span (Sibel *et al.*, 2002). The different used substrates finds utility as fertilizers and soil conditioners for the growth of plants (Brenneman *et al.*, 1994) and also as animals feed (Soto *et al.*, 1999). The present investigation aims to evaluate the nutritional and bioactive components of local strain of *P. cystidiosus* collected from Patiala growing on the living stem of *Lagerstroemia speciosa*.

## MATERIAL AND METHODS

### Source of culture

Viable mycelia culture was raised through tissue culture of mushroom tissue taken from the confluence point of pileus and stipe of *P. cystidiosus* sporophores picked from the living stem of *Lagerstromia speciosa* growing along roadsides near Girls Hostel on the campus of Punjabi University, Patiala. The pure mycelia culture of this mushroom has been deposited in the ICAR-DMR Culture Bank under accession number DMRP-394. The raised pure culture was aseptically inoculated on PDA slants and subsequently incubated at  $28 \pm 2^\circ\text{C}$  in the incubator.

### Production of sporophores and Sample preparation

The sporophores of *P. cystidiosus* were raised on the wheat straw based substrate formulations consisting of 9 parts of wheat straw and 1 part of rice husk. The sporophores so obtained were air dried in lyophilizer (Ly-christ Alpha 1-2) and powdered. The extraction of dried mushrooms sample (5g) was done using 100 mL of methanol in rotary shaker (150 rpm) at  $25 \pm 1^\circ\text{C}$  for 24 hours. The extract was filtered through Whatman filter paper Number 4. Two additional 100 mL portions of methanol were added to the left over residue. The combined methanolic extract was evaporated at  $40 \pm 1^\circ\text{C}$  to dryness and re-dissolved in methanol at a concentration of 50 mg/mL and stored at  $4^\circ\text{C}$ .

### Chemicals and reagents

The various chemicals used during the present study include acetic anhydride, acetone, aluminium chloride, chloroform, 1,4-dichlorobenzene, ethyl acetate, methanol, petroleum ether, sodium hydroxide, sulphuric acid which were purchased from LobaChemie Pvt. Ltd. India while calcium carbonate,

calcium sulphate, diosgenin, sodium carbonate and quercetin were purchased from Himedia Laboratories Pvt. Ltd. India.

### Experimental design

All the experiments were conducted under aseptic conditions in Mycology and Plant Pathology Laboratory, Department of Botany, Punjabi University, Patiala and arranged in a randomized complete design with three replicates per treatment.

### Methodology for determination of dry matter, proximate composition and energy value

The methanolic extract of *P. cystidiosus* was used for the qualitative estimation of nutritional components by applying standard methods (Harborne, 1973; Trease and Evans, 1989; Kokate, 2004). The dry matter in the mushroom sample was estimated by subtracting the moisture content from the fresh weight of fruiting bodies. The proximate composition was analyzed following AOAC (1995) protocols. The total protein content was determined by methods of Lowry *et al.* (1951). Crude fat was estimated with petroleum ether by extracting a known weight of sample and crude fiber content was determined by acid-alkali digestion of mushroom sample by following the protocol given by Maynard (1970). The ash content was determined by igniting the known weight of sample at  $525^\circ\text{C}$  for 4 hours. The moisture content was determined by re-heating the dried sample at  $105^\circ\text{C}$  for 16 hours until a constant weight was obtained. Total carbohydrates (Raghuramulu *et al.*, 2003) and energy value (FAO, 2002) was calculated by applying following formulation:

Total carbohydrates =  $100 - (\text{g of moisture} + \text{g of proteins} + \text{g of fats} + \text{g of ash} + \text{g of fiber})$

Energy value (Kcal/100g) =  $\text{proteins} \times 4 + \text{fats} \times 9.10 + \text{carbohydrates} \times 4.2$ .

**Qualitative estimation of bioactive compounds**

For the qualitative estimation of phenols, flavonoids, steroides and alkaloids, protocol given by Harborne (1973), Trease and Evans (1989) and Kokate (2004), respectively were followed.

**Quantification of total phenolics (Singleton and Rossi, 1965)**

For the determination of total phenolics, 1 mL of mushroom sample (methanolic extract) was mixed with 1mL of Folin ciocalteu's reagent. The reaction mixture was kept for 3 minutes, after which 1 mL of sodium carbonate solution was added. The test tubes were placed in the dark for 90 minutes after which absorbance was read at 725nm. Gallic acid was used as standard and results were expressed as mg of Gallic acid equivalents per g of extract.

**Quantification of total flavonoids (Yoo *et al.*, 2008)**

The methanolic extract sample (250 mL) was mixed with 1.25 mL of distilled water and 75  $\mu$ L of 5% NaNO<sub>2</sub> solution. After 6 minutes of incubation, 1M NaOH (500 mL) solution and 275  $\mu$ L of distilled water was added at 510 nm. Rutin was used as standard and the results were expressed as mg of rutin perg of sample.

**Quantification of  $\beta$ -carotene and lycopene (Barros *et al.*, 2007)**

The dried methanolic extract of mushroom (100g) was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for the quantitative estimation of  $\beta$ -carotene and lycopene. The solution was filtered through Whatman number 4 filter paper. The absorbance of filterate was measured at 453, 505 and 663 nm. The contents were calculated by using the following equation:

$$\beta\text{-carotene (mg/100mL)} = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

$$\text{lycopene (mg/100mL)} = -0.0458 \times A_{663} + 0.372 \times A_{505} - 0.0806 \times A_{453}$$

The results were expressed as  $\mu$ g of carotenoid per g of extract.

**Quantification of Alkaloids (Maxwell *et al.*, 1995)**

For the estimation of alkaloids, 5g of dried powder of mushroom was extracted with 100 mL of 10% acetic acid. The reaction mixture was left to stand for four hours. The extract was filtered through Whatman filter paper number 4 to remove the debris. To this filtrate, 1% ammonium solution was added drop wise until the precipitates were formed. The alkaloids thus obtained were dried at 65°C in an oven. The percentage of alkaloid was calculated by using following formula:

$$\text{Percentage alkaloids (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

**Quantification of steroids (Okeke and Elekwa, 2003)**

For the determination of steroid content, 0.1g of mushroom powder sample was weighed and mixed with 10 mL of ethyl acetate. The reaction mixture was allowed to stand for 2 hours with occasional shaking followed by filtration. To 1 mL of this reaction mixture, 1 mL of chloroform was added. This mixture was then treated with 1.5 mL of ice cold acetic anhydride followed by addition of 2 drops of concentrated sulphuric acid. The absorbance was measured at 420 nm. The diosgenin was used as standard for the preparation of calibration curve (0.01- 0.1 mg/mL) and the steroid content was expressed as mg of diosgenin equivalents per g of dry weight of mushroom sample.

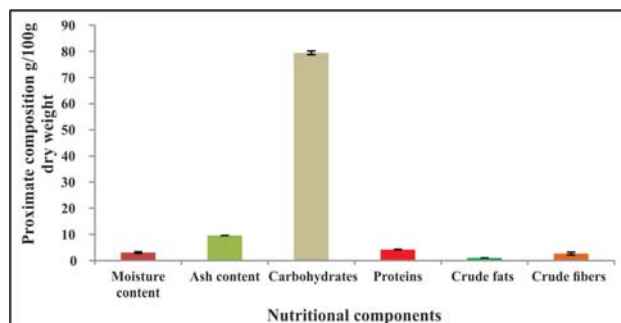
## RESULTS

The results of the nutritional and nutraceutical profiles of *P. cystidiosus* are presented below:

### Nutritional profile of *P. cystidiosus*

The mushroom sample was evaluated both qualitatively as well as quantitatively for the proximate components present in the mushroom. The qualitative chemical examination of methanolic extract was done for the screening of carbohydrates, reducing sugars, proteins and fats. A positive indication for the presence of the evaluated constituents was noted during qualitative examination. The results of chemical screening of the prepared extracts are presented in **Table 1**. The results of the proximate analysis of the evaluated mushroom sample are depicted in **Table 2** and **Figure 1**. The evaluated sample of *P. cystidiosus* revealed the presence of 3.08% moisture content, 9.61% ash content, 79.45% carbohydrates, 4.2%

proteins, 1% crude fats, 2.66% crude fibers and energy value of 343.62 kcal/100g of dry sample.



**Fig. 1.** Histogram showing different nutritional components (g/100g) in *P. cystidiosus* on dry weight basis

### Neutraceutical profile of *P. cystidiosus*

The mushroom sample was evaluated both qualitatively as well as quantitatively for its nutraceutical components. The qualitative chemical examination of methanolic extract of *P. cystidiosus* was done for the screening of flavonoids, phenols,

**Table 1.** Chemical screening of methanolic extract of *P. cystidiosus*

S. No.	Phytoconstituent	Chemical Test	Methanol extract of <i>P. cystidiosus</i>
1	Carbohydrates	Anthrone reagent test	++
2	Reducing sugars	Picric acid test	++
3	Proteins	Biuret test	++
4	Fats	Spot test	++

++ Presence

**Table 2.** Nutritional components of *P. cystidiosus* (g/100g) of dry sample

S. No.	Nutritional component	<i>P. cystidiosus</i>
1	Moisture Content (%)	3.08 ± 0.30
2	Ash Content (%)	9.61
3	Carbohydrates (%)	79.45 ± 0.70
4	Proteins (%)	4.2 ± 0.04
5	Crude fats (%)	1 ± 0.0
6	Crude fibers (%)	2.66 ± 0.57
7	Energy Value (Kcal)	343.62 ± 2.80

alkaloids and steroids. The result of chemical screening of prepared extract is given in **Table 3**. The results obtained during the estimation of different bioactive components are depicted in **Table 4** and **Figure 2**. On dry weight basis, nutraceutical components of *P. cystidiosus* revealed the presence of phenols (0.97%), flavonoids (1.1%), steroids (0.14%), and alkaloids (0.93%). The estimated value of  $\beta$ -carotene and lycopene was 1.02 and 0.44  $\mu$ g/g, respectively on dry weight basis of mushroom sample.

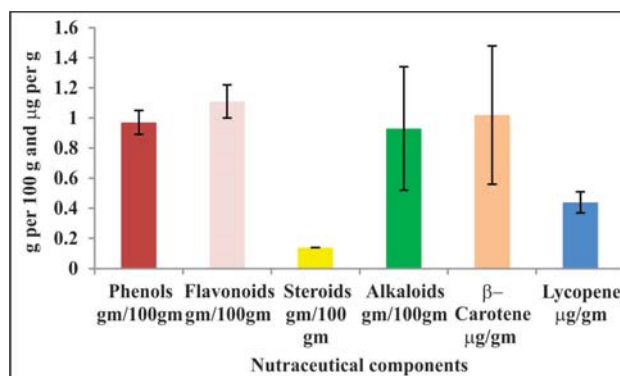
**Table 3.** Chemical screening of methanolic extract of *P. cystidiosus*

S. No.	Phytoconstituent	Chemical Test	Methanol extract of <i>P. cystidiosus</i>
1	Flavonoids	Alkaline reagent test	++
2	Phenols	Folin- Ciocalteu test	++
3	Alkaloids	Tannic acid test	++
4	Steroids	Salkowski's test	++

++ Presence

**Table 4.** Nutritional components of *P. cystidiosus* (g/100g and µg/g) of dry sample

S. No.	Nutritional component	<i>P. cystidiosus</i>
1	Phenols (%)	0.97 ± 0.08
2	Flavonoids (%)	1.11 ± 0.11
3	Steroids (%)	0.14 ± 0.0
4	Alkaloids (%)	0.93 ± 0.41
5	β- Carotene (µg/g)	1.02 ± 0.46
6	Lycopene (µg/g)	0.44 ± 0.07

**Fig. 2.** Histogram showing different nutraceutical components (g/100g and µg/g) in *P. cystidiosus* on dry weight basis

## DISCUSSION

The variation in different constituents present in mushroom is reported to be dependent on various factors including surrounding environment, temperature, relative humidity during growth and relative amount of water absorbed during storage, substrate composition, etc (Crisan and Sands, 1978). The proportion of moisture present in the mushroom sample and the overall composition of dry matter are also very important since these are reported to influence the nutrient composition of mushrooms (Ouzouni *et al.*, 2009). The documented value of the moisture content in a mushroom species is reported to range between 8-14%. As compared in the presently evaluated sample of mushroom comparatively lower value for moisture (3.08%) was estimated on dry weight basis. As reported by Crisan and Sands (1978), this may be primarily due to the lesser amount of moisture retained in the mushroom sample under dry conditions. The mushroom dry matter constitute ash which is a composite mixture

containing different impurities of carbonates, oxalates and silicates besides the normal constituents as are revealed during the evaluation of its proximate composition. In the presently evaluated mushroom sample the presence of 9.61% ash content has been estimated which is almost comparable to the proportion ash content range (5.40-8.40g/100g dry matter) reported by Ragunathan and Swaminathan (2003) while working with different *Pleurotus* species. This variation has been attributed to the varied composition of the substrates used for their cultivation.

Carbohydrate is a major constituent of mushrooms which is mainly made up of glucose, mannitol, á-trehlose and glycogen as reserve polysaccharides (Shweta *et al.*, 2014). As is apparent from the data presented in Table 2 and Figure 1, total carbohydrate content in the presently evaluated indigenous strain of *P. cystidiosus* is substantially high (79.45%) as compared to the amount of carbohydrate (40.1 to 42.2g/100g) reported to be present in different species

of *Pleurotus* (Ragunathan and Swaminathan, 2003). While working with *Pleurotus sajor-caju*, Shweta *et al.* (2014) evaluated 62.97g/100g of total carbohydrates which is also much less in comparison to the value of carbohydrate evaluated presently (79.45%). Otherwise the species of genus *Pleurotus* are considered to be a good source of carbohydrates (Khan and Tania, 2012), the statement which is in agreement with the present observation. The most important group of macromolecules which contribute to the nutritive value of mushrooms is its proteins (Chang and Hayes, 1978) which is reported to depend upon the genetic constitution of the individual species, physical and chemical properties of the growing media, substrate composition, pileus size and the time of harvest (Alam *et al.*, 2008; Akyuz and Kirbag, 2010). In different *Pleurotus* species substantially high amount of protein ranging from 11-44.3g/100g of dry weight has been documented by different investigators (Ragunathan and Swaminathan, 2003; Alam *et al.*, 2008; Khan *et al.*, 2008; Akyuz and Kirbag, 2010; Mshandete and Cuff, 2007; Dundar *et al.*, 2008; Gbolagade *et al.*, 2006; Akindahunsi and Oyetayo, 2006; Kadiri *et al.*, 1990; Khanna *et al.*, 1992; Patil *et al.*, 2010 and Oyetayo *et al.*, 2007). However, as compared in the presently evaluated local strain of *P. cystidiosus* the proportion of net protein content is much less (4.2%). The results depicted here are in conformity with the previous reports of Yang *et al.* (2001) that abalone winter oyster mushroom contained lesser amount of proteins. Fat is another constituent of mushrooms which is reported to help in the digestion of food and also act as a source of energy (Tocher, 2003). The results in this regard in the present study are in conformity with the earlier reports. The presently evaluated mushroom sample contain low amount of fats (1g/100g of dry weight) which is similar to the reports given by Shweta *et al.*, 2014 while working with *P. sajor-caju* (0.91g/100g of dry weight). In different species of genus *Pleurotus* grown on different substrates the amount of fat content

is reported to range from 0.95-3.16g/100g of dry matter (Ragunathan and Swaminathan, 2003), which is comparable to the presently evaluated value of fat in *P. cystidiosus*. Fiber is also an important component of the diet. It consists of non-digestible carbohydrate polymers that may be associated with other non-carbohydrate components (Elleuch *et al.*, 2011). The crude fiber content in *P. cystidiosus* has been evaluated at 2.66g/100g on dry weight basis which is comparable to the value of 2.40 g/100g of dry weight as reported by Roy (2015) while studying *Pleurotus ostreatus*. Guardia (2005) also reported fiber content of 3.0-4.5g/100g in different *Pleurotus* species, however the per cent crude fiber content in the presently evaluated mushroom is slightly on the lower side (2.66 g/100g on dry weight basis). The energy value of *P. cystidiosus* cultivated on wheat straw was calculated at 343.62 kcal/100g of dry matter. These values are identical with the energy value of *P. sajor-caju* (325.85 kcal/100g of dry matter) as reported by Shweta (2014). In different *Pleurotus* species also energy value ranging from 272-316 kcal/100g of dry matter has been reported by Ragunathan and Swaminathan (2003). The presently calculated energy value also falls near to the upper value in this range.

The phytochemicals with bioactive potential have been reported in number of mushroom species (Asuquo and Etim, 2011; Adebayo *et al.*, 2012; Afiukwa *et al.*, 2013 and Unekwo *et al.*, 2014). The phytochemical constituents evaluated on qualitative basis; including phenols, flavonoids, alkaloids and steroids; in the cultivated local strain of *P. cystidiosus* are depicted in Table 3. The results of present study are in agreement with the previous such reports from elsewhere. Adebayo *et al.* (2012) while working with *P. pulmonarius* documented the presence of alkaloids, saponins, steroids, flavonoids and anthraquinones. The amount of total phenols, flavonoids, steroids, alkaloids,  $\beta$ -carotene and lycopene

presently documented in *P. cystidiosus* are depicted in Table 4 and Figure 2. On quantitative basis the total phenolic content estimated was 0.97g/100g of dry matter, which is slightly on the lower side as compared to the phenolic content reported on dry weight basis in *P. cystidiosus* (1.02g/100g) and *P. ostreatus* (1.5g/100g) by Yanga *et al.* (2002). However, Kalaw and Albinito (2014) evaluated much less amount of phenolic content (0.34 g/100g of dry matter) in *P. cystidiosus* in comparison to the presently evaluated local strain. Another antioxidant component evaluated presently in the mushroom sample on dry weight basis was total flavonoid (1.11g/100g). In comparison Shweta *et al.* (2014) while working with *P. sajor-caju* reported much less amount of flavonoid (0.4g/100g) on dry weight basis. Juma *et al.* (2016) also reported only 0.03 g/100g of flavonoid content in the edible species of *P. cystidiosus* from Tanzania. Muruke (2014) also evaluated the total flavonoid content of *P. cystidiosus* which was reported to range from 5-31.64 mg/100g of dry weight. The alkaloid content of studied mushroom was 0.93g/100g which is on the lower side.

The lesser value of alkaloids adds to the culinary properties of the mushroom. Steroids were also found to be present in the mushroom sample in small proportion (0.14g/100g). During the present study, the content of  $\beta$ -carotene and lycopene was 1.02 $\mu$ g and 0.44 $\mu$ g/g of dry weight, respectively. The results of the present study indicates the lesser values of  $\beta$ -carotene and lycopene in the local strain of *P. cystidiosus* as compared to the amount of  $\beta$ -carotene (2.49-3.81 mg/100g) and lycopene (2.16-3.10 mg/100g) content present on dry weight basis in *Lentinus* species (Hussein *et al.*, 2015). Also the findings of Muruke *et al.* (2014) revealed more amount of  $\beta$ -carotene content (2-45mg/100g) in *P. cystidiosus* as compared to the amount of  $\beta$ -carotene in the presently evaluated local strain. It is a well established fact that natural antioxidants are of considerable interest as

dietary supplements or food preservatives (Jayakumar *et al.*, 2006). Mushrooms because of the presence of such components as Phenoles, Alkaloids,  $\beta$ -carotene, Lycopene, etc. are important source in view of which presently also an attempt was made to determine the antioxidant components in the methanolic extract of *P. cystidiosus*. Similar such observations are reported by Shweta (2014) while working on mushrooms. For the synthesis of vitamin A, carotene is used as a precursor molecule which also acts as an antioxidant (Ross *et al.*, 2011), while lycopene is known as the significant singlet oxygen quencher compared with a variety of carotenoids and  $\alpha$ -tocopherol *in vitro* (Tibuhwa, 2014). Presently evaluated mushroom contain all such nutraceutically important components hence qualifies for consideration as dietary supplement (Chang and Miles, 2008).

## CONCLUSION

The evaluated local strain of *Pleurotus cystidiosus* is quite rich in nutritional and nutraceutical components as is apparent from the qualitative and quantitative analysis of its dried sample. In view of this it fits very well into the category of dietary supplements. At the same time the presence of low fat and high fiber content in this mushroom make it a health food for the persons suffering from heart diseases and diabetes. The antioxidant potential of the mushroom components makes it suitable to be categorized amongst the functional foods with variety of health benefits

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