Bioprospecting of selected wild mushrooms from Jharkhand, India

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Abstract

Nine different varieties of mushrooms were collected from the various niches of Ranchi, Jharkhand, India. These mushrooms were identified on the basis of their macroscopic and microscopic studies. After identification, these mushrooms were found to be *Agaricus bisporus*, *Agaricus campestris*, *Ganoderma lucidum*, *Pleurotus populinus*, *Pleurotus pulmonarius*, *Amanita pantherina*, *Astraeus hygrometricus*, *Russula emetica* and *Russula cyanoxantha*. For all these 9 species of mushrooms bioprospecting with reference to nutritional, toxicological and medicinal aspects were carried out. At present, nutritional aspects are being reported. For nutritional aspects these mushrooms were analyzed for their different properties like carbohydrate, protein, fat, vitamins and mineral contents. In the present study, carbohydrate content was highest in *R. emetica* (34.34 g/100 g dw) and lowest in *P. populinus* (8.18 g/100 g dw). The protein content was found to be highest in *G. lucidum* (48.86 g/100 g) and lowest in *Amanita pantherina* (13.59 g/100 g dw). Fat content was detected to be highest in *Astraeus hygrometricus* (5.20 g/100 g dw) and lowest in *P. populinus* (0.113 g/100 g dw). Riboflavin content was highest in *G. lucidum* (18.37 g/100 g dw) and almost absent in *R. cyanoxantha* (0.018 g/100 g dw). Content of ascorbic acid was found to be highest in *A. bisporus* (27.15 g/100 g dw) and lowest in *P. pulmonarius* (13.38 g/100 g dw). Highest amount of Ca was detected in *P. pulmonarius*. Level of K, Mg and Na was seen to be highest in *A. bisporus*.

Keywords

carbohydrate, mushrooms, protein, vitamins

Introduction

Mushroom is the fleshy fungi of the class Basidiomycota, usually having an umbrella shaped cap borne on a stalk with gills on the underside of the cap especially any of the edible kinds. A fungus is the second most diverse of all groups and is considered as a prime member of the other mega-diverse groups like insects, bacteria, arachnids and nematodes (1). Technically speaking macrofungi that are mostly scattered throughout Ascomycota, Basidiomycota and Zygomycota are defined as those fungi which bear large, easily observed spore-bearing structures that are formed above or below ground. A recent global study regarding macrofungal diversity estimated it to be within the range of 53000 to 110000 species (2).

Mushrooms are a rich source of proteins, vitamins and minerals and low in fat content (2-8%) and this unique chemical constitution of mushrooms makes them low calorie food and cheese diet for those suffering from...
Shorea robusta, Lentinus pestris, Ganoderma lucidum, Pleurotus populinus, Pleurotus they were found to be the basis of these study mushrooms were identified and the Department of Bioengineering, BIT mesra, Ranchi. On their identification purpose. Voucher specimens leaves. Macroscopic and microscopic studies were carried out for their identification during rainy season (June - September) are collected by local people for their consumption and also serve as a source of additional earnings. Botanists have tried to identify these mushrooms (9). But so far, none of their work has been related with bioprospecting with reference to nutritional, toxicological and medicinal aspects. Therefore, the present work has been undertaken. Mushrooms from the various areas of Ranchi district were collected and were analyzed for their nutritional, toxicological and medicinal properties. In this paper, we are reporting the bioprospecting of local mushrooms (BIT Mesra, campus Sal forest and other niches of Ranchi district) with reference to nutritional aspects. Bioprospecting of local mushrooms with reference to medicinal aspects has been published earlier (10).

Materials and Methods

Collection of mushrooms

Mushrooms were collected from various niches of Ranchi district. Collecting mushrooms for identification requires a pocket knife, a Sharpie and a basket. A pocket knife is needed in order to dig up the bases of some mushroom. A wooden stick to search between the vegetation and dead leaves. Macroscopic and microscopic studies were carried out for their identification purpose. Voucher specimens were deposited in the herbarium of the microbiology lab in the Department of Bioengineering, BIT mesra, Ranchi. On the basis of these study mushrooms were identified and they were found to be Agaricus bisporus, Agaricus campestris, Ganoderma lucidum, Pleurotus populinus, Pleurotus pulmonarius, Amanita pantherina, Atraceus hygrometricus, Russula emetica and Russula cyanoxantha.

Extraction of mushroom

Extraction was carried out by the method followed by Smidsgaaard (11) with some modifications. 18 g of mushrooms sample was taken and was crushed. It was then mixed in 36 ml of solvent I containing of methanol: dichloromethane: ethyl acetate in 1:2.3 proportion. It was then left overnight at 5°C. Then after centrifugation supernatant and the remaining pellet was evaporated and was then mixed in solvent II, consisting of methanol (43.76 ml), hydrochloric acid (0.04 ml), formic acid (1.2 ml) and water (5 ml).

Determination of carbohydrate

Carbohydrate determination was carried out by Anthrone test (12) with some modifications. Firstly, for the standard curve of sucrose, anthrone reagent was prepared by mixing 50 mg of enthrone in 25 ml of ice cold sulphuric acid. Then standard solutions of sucrose were made from stock solution of 0.1 mg/ml. The concentration was prepared as 0.02, 0.04, 0.06, 0.08, 0.1 mg/ml. Then 2 ml of anthrone reagent was added to test tubes and their colour was changed to blue green. Then test tubes were incubated for 10 min in boiling water bath. Then OD was taken at 620 nm. For estimation of sugar in a mushroom sample about 200 µl of methanolic extract of the sample was mixed with 800 µl of distilled water and 2 ml of anthrone reagent and then OD was taken at 620 nm. For blank 1 ml of distilled water was mixed with 2 ml of anthrone reagent.

Determination of protein

It was carried out by Lowry method (13) with some modifications. Estimation of protein in mushroom sample was done by mixing 0.5 ml of methanolic extract in 0.5 ml of DW. Then, 5 ml of Reagent I (sodium carbonate dissolved in 0.1N sodium hydroxide and 1% sodium, potassium tartrate and 0.5% copper sulfate) was added and the mixture was allowed to incubate at RT for 15 minutes. Then, 0.5 ml of Reagent II (1 part Folin-Phenol [2 N]: 1 part water) was mixed and the mixture was again incubated for 30 min in the dark. Finally, OD was taken at 660 nm. Standard curve of BSA (Bovine Serum Albumin) was used as a reference.

Determination of fat

Amount of fat was determined through a method (14) with some modifications. 2.5 g of mushroom sample was suspended in 25 ml of chloroform: methanol (2:1 v/v) mixture and was mixed thoroughly and allowed to stand for 3 days. The solution was then filtered. After filtration, it was centrifuged at 4000 rpm for 10 min. The upper layer containing methanol was removed by pipette and chloroform was evaporated by heating. Remaining portion was having the crude lipid.

Determination of riboflavin

It was done by the standard method (15) with some modifications. Sample powder (1.25 g) was mixed with 375 ml of water and 1.25 ml of glacial acetic acid. After boiling for 2 min, the solution was then cooled. Then, 7.5 ml of 1.0 M sodium hydroxide solution was added and diluted to 137.5 times.
ml with distilled water. The solution was filtered. OD was taken at 444 nm. For blank water was used. Amount of riboflavin was calculated as follows:

\[
\text{Riboflavin (mg) / 100 gm of the sample} = \frac{SA \times 500}{328 \times 10000} \times \text{SW}
\]

**Determination of ascorbic acid**

The standard curve of ascorbic acid was prepared by the standard method (16) with some modifications. Varying concentrations were made from 3 mg/ml of stock solution of L-Ascorbic acid. Finally, OD was taken at 521 nm. Amount of ascorbic acid was estimated in sample by mixing 100 mg of sample with 10 ml of 1% metaphosphoric acid and was kept for 45 min at RT. Then, it was filtered. Then, 1 ml of filtrate was mixed with 9 ml of 2,6DCPIP and was then incubated for 30 min. Finally, OD was taken at 515 nm (55).

**Mineral and trace elements determinations**

For the purpose of determining mineral elements (K, Na, Mg, Ca and Cr), trace elements (Fe, Cu, Mn and Zn) and toxic heavy metal (Pb), samples of mushrooms were dried and then were digested in concentrated HNO\(_3\) and H\(_2\)O\(_2\) (18). Then all the minerals were quantified by ICP-OES (Perkin Elmer, USA; Optical 2100DV).

**Statistical analysis**

It was done by using the MS Excel worksheet. The error bars in the plots are representing the standard deviation. Analysis of data was carried out using R version 3.4.0 – “You Stupid Darkness” for Duncan’s Multiple Range Test (DMRT). Using DMRT differences among means was calculated at 5% level of significance.

**Results and Discussion**

In recent years, mushrooms have earned interest on account of their pharmacological characteristics and their high-protein/low-fat nutritional value (19). But, mushrooms have not been screened on a large scale for the presence of active components. The carbohydrate, protein, fat, vitamins and mineral contents have been investigated and such investigations showed that all the mushrooms are rich in protein, vitamins and minerals. It was also found that these mushrooms had very less fat content so they are very healthy and since all mushrooms have been collected from local forests so they are cheap too. Different kinds of vitamins like vitamin B2 and vitamin C have been found in these mushrooms.

Carbohydrate content was found to be low in all the collected samples, which shows that these mushrooms are a low calorie food. In the present study, carbohydrate content was highest in *R. emetica* and lowest in *P. populinus*. Glucose and mannitol are the two important digestible carbohydrates and both of them are present in low quantities (not more than 1% of the dry weight) in *Agaricus bisporus* (20). In collected samples of *Agaricus bisporus*, carbohydrate content was found to be (17.05 g/100 g dry weight (dw)) approximately same as reported earlier by Manzi et al. (17.02 g/100 g dw) (21). But the amount of carbohydrate was lesser than observed value 74.01 g/100 g dw (22) and 26.29 g/100 g dw (23). Also, it was found that the carbohydrate content in the cultivated species of *A. bisporus* to be 4.5 g/100 g and it was less than our result (24). In the studied sample of *A. campestris*, carbohydrate content was found to be 22.72 g/100 g dw and it was lesser than observed values 58.16 g/100 g dw (25) and 58.1 g/100 g dw (22). In this study, carbohydrate content in *G. lucidum*, was found to be 13.42 g/100 g dw and it was less than observed (40.14 g/100 g dw) (26). MDidea website (27) reported 43.1 g carbohydrate/100 g dw (28) reported 38.04 g/100 g dw in *G. lucidum*. In collected sample of *P. pulmonarius*, carbohydrate content was found to be 12.76 g/100 g dw and it was lesser than as observed 43.40 g/100 g dw (29).

Also, when compared with the cultivated species of *P. pulmonarius* as observed (30), carbohydrate content was found to be 43.40 g/100 g dw which is more than what is found in our study. In this study, carbohydrate content in *Astraeus hygrometricus* was found to be 29.61 g/100 g dw and it was approximately same as observed value 29.48 g/100 g dw (31, 32). In the present study, carbohydrate content in *R. cyanoxantha* was found to be 25.43 g/100 g dw and it was less than reported value 74.6 g/100 g (22). Also, it was (33) observed that very low amount of carbohydrate i.e. 9.56 g/100 g dw in *R. cyanoxantha*. In collected sample of *R. emetica*, the amount of carbohydrate was found to be 34.34 g/100 g dw and it was higher than observed 27.09 g/100 g dw (33).

Proteins are a very important nutrient in foods. All studied mushrooms were found to be the good source of proteins that is why they are also said as "meat of pov-
In this study, protein content in *G. lucidum* was detected to be 48.86 g/100 g dw and it was more than observed 20.61 g/100 g dw (26) and 17.12 g/100 g dw (28). In the present study, the amount of protein in *P. pulmonarius* was 34.32 g/100 g dw and it was more than the cultivated one as reported value 20.03 g/100 g dw (39). Also, it was more than cultivated species of *Pleurotus* as observed 7.06 g/100 g dw (40). Protein content in *P. pulmonarius* as observed (30) was 37.63 g/100 g dw which is approximately same what we got in our study. In the studied sample of *Astraeus hygrometricus* protein content was found to be 17.57 g/100 g dw and it was higher than observed 11.71 g/100 g dw (31, 32). In collected sample of *R. cyanoxantha* protein content was 27.46 g/100 g dw and it was more than in the other report (22) who found the protein content to be 16.8 g/100 g dw (33) found to be 49.20 g/100 g dw. According to another observations made (33) protein content in *R. emetica* was found to be 33.24 g/100 g dw and it was higher than what we got in our studied sample (19.46 g/100 g dw).

Mushrooms have very low fat content so they serve as a healthy source of food. In this study, fat content was detected to be highest in *Astraeus hygrometricus* and lowest in *P. populinus*. In collected sample of *A. bisporus*, crude fat content was 3.93 g/100 g dw and it was higher than observed value 0.33 g/100 g (21) and 2.12 g/100 g dw (29). But, as observed (41) crude fat content was 1.66 g/100 g dw (35) and it was 2.28 g/100 g dw which shows that fat content in our sample of *A. bisporus* is in higher amounts. Also, the amount of crude fat in cultivated species of *A. bisporus* as observed (24) was 0.33 g/100 g fw and it was lesser than what we got in our study. In the present study, crude fat content in *A. campestris* was found to be 1.58 g/100 g dw and it was lesser than as observed 2.32 g/100 g dw (42), 2.7 g/100 g dw (38) and approximately same as the value i.e 1.1 g/100 g dw (22). But, it was higher than as observed value 0.11 g/100 g dw (25). In the studied sample of *G. lucidum*, crude fat was 1.34 g/100 g dw and it was in a lesser amount than observed value 3.2 g/100 g dw (28). In this study, the amount of crude fat in *P. pulmonarius* was found to be 0.66 g/100 g dw and it was lesser than observed value 4.32 g/100 g dw (40) and 1.93 g/100 g dw (30). In the studied sample of *Astraeus hygrometricus* crude fat content was 5.20 g/100 g and it was higher than as observed 2 g/100 g dw (43). In this study, crude fat content in *R. cyanoxantha* was found to be 3.61 g/100 g dw and it was found to be in a higher amount than the other observed value like 1.52 g/100 g dw made (22) but it was lesser than observed 7.87 g/100 g dw (33). In collected sample of *R. emetica*, crude fat content was found to be 1.56 g/100 g dw and it was lesser than as observed value 3.94 g/100 g dw (33).

Vitamins like B2 and C were analyzed for these collected mushrooms. For riboflavin, values ranged from 0.001 to 18.37 mg/100 g dw, and for ascorbic acid from 13.38 to 27.15 mg/100 g dw. In the present study, Riboflavin content was highest in *G. lucidum* and almost absent in *R. cyanoxantha*. Vitamin C is one of the key contributors to the antioxidant activity of the fruits, vegetables and mushrooms. All the studied mushrooms were rich in vitamin C and hence, all of them are a good source of antioxidant. Ascorbic acid content was found to be highest in *A. bisporus* and lowest in *P. pulmonarius*. In collected sample of *A. bisporus*, vitamin B2 and vitamin C content were detected to be 3.93 mg/100 g and 27.15 mg/100 g dw and it was found that vitamin B2 was lesser but vitamin C was higher than the observations made (24) who found that in cultivated species of *A. bisporus* vitamin B2 content was 5.1 mg/100 g dw and vitamin C was 17 mg/100 g dw. Also, the amount of vitamin B2 in *A. bisporus* was found to be more than recorded value 0.27 mg/100 g dw in cultivated one as observed (44). In the studied sample of *A. campestris* vitamin B2 was found to be 5.07 mg/100 g dw and vitamin C was found to be 20.94 mg/100 g dw and both of them were higher than observed by Anderson (45) who found vitamin B2 to be 0.52 mg/100 g dw and vitamin C to be 8.60 mg/100 g dw. In this study, vitamin B2 content in *P. pulmonarius* was found to be 0.69 mg/100 g dw and vitamin C was 13.38 mg/100 g dw. But, when compared with the cultivated one vitamin B2 and vitamin C were higher than observed (46). According to whom vitamin B2 was 0.26 mg/100 g dw and vitamin C was 6.09 mg/100 g dw (40) found vitamin B2 to be 0.066 mg/100 g dw and vitamin C to be 45.33 mg/100 g dw respectively. In collected sample of *Astraeus hygrometricus*, vitamin C was found to be 15.68 mg/100 g dw and it was higher than observed value 3.26 mg/100 g dw (32). In the studied sample of *R. cyanoxantha*, vitamin C was found to be 21.9 mg/100 g dw and it was more than in reported one (47).

Several major, minor and toxic minerals have been found in these collected mushrooms. In the present study,
Fig. 5. Vitamin C content in the collected mushroom samples

all of the mushrooms have shown the presence of good amount of potassium, calcium and iron. Among, all the minerals potassium was found to be in highest amount. As a result of this, these mushrooms could serve as a good source of potassium and can be used against problems like, renal and myocardial damage which are caused due to potassium deficiency (48, 49). The highest amount of Ca was detected in P. pulmonarius. Level of K, Mg and Na was seen to be highest in A. bisporus which is in accordance with the original findings (50, 51, 52). The highest level of Cu and Fe was seen in Amanita pantherina. Level of Mn was detected to be highest in Atrasues hygrometricus. In collected samples of A. bisporus, minerals like, Ca, K, Mg, Na and Fe were higher in amount, but minerals (like, Cu, Mn, Zn) were lesser than reported (18). But, on the other hand, minerals such as Ca, K, Mg, Na and Fe were found lesser than as observed (53). Also, amount of Mg was found to be more than reported (54). Level of Fe was detected lesser than as observed (55). As compared to the cultivated species, minerals (like, Fe, Cu and Zn) were lesser in amount than in our studied sample of A. bisporus (56). In the studied sample of A. campestris, some minerals like, Ca and Na were more but some minerals like, K, Mg, Fe were lesser than reported (53). Also, Mn was found to be very less than observed (54). In this study, with respect to studied a sample of G. lucidum, minerals such as CA, K, Mg, Na and Fe were lesser than reported (57). In the present study, in P. pulmonarius minerals (like, Mg, Fe, Ca, Mn, Cr) were seen to be lesser than the cultivated species as observed (58). In the collected sample of Atrasues hygrometricus, minerals (like, Ca, K, Mg) were lesser than observed (30). Also, minerals (like, P, K, Ca, Fe) were detected to be lesser than as observed (31). In collected sample of R. cyanoxantha, minerals such as Cu, Zn and Mn were lesser than as observed (59).

The variations in the amount of carbohydrate, protein, fat, vitamin B2, vitamin C and different minerals in all the samples tested may be due to variation in soil and climatic conditions. According to the observations made, it was found that changing the substrates for cultivating mushrooms leads to the change in the nutritional composition of those mushrooms (38, 60). Also, it was observed that mushrooms show different amount of minerals when grown in different types of soils (59). Thus, it can be concluded that the nutritional composition of any mushroom depends on the media composition and the climatic conditions in which it is being grown.

Among all the mushroom studied, G. lucidum and P. pulmonarius was found to be healthy as it was low in carbohydrate and fat content and high in protein, vitamin B2 and mineral content. Also, no reported papers were found on nutritional composition of P. pulmonarius, hence, it was a new one to be studied.

The Ranchi district of Jharkhand has diverse geographical niches as it is located on chotanagpur plateau. The plateau is full with diverse forest resources. These forest areas harbour many species of mushrooms, which has not been explored scientifically. In the present work, nine different samples of mushrooms were collected from different areas of Ranchi district. These mushrooms were found to be very healthy, as they contained low carbohydrate and fat. All the collected mushrooms were found to be the good source of protein, vitamins and various minerals.
Conclusion

The Ranchi district of Jharkhand has diverse geographical niches as it is located on chotanagpur plateau. The plateau is full with diverse forest resources. These forest areas harbor many species of mushrooms, which has not been explored scientifically. In the present work, nine different samples of mushrooms were collected from different areas of Ranchi district. These mushrooms were found to be very healthy, as they contained low carbohydrate and fat. All the collected mushrooms were found to be the good source of protein, vitamins and various minerals. In the present study, carbohydrate content was highest in R. emetica (34.34g/100g dw) and lowest in P. populinus (8.18g/100g dw). The protein content was found to be highest in G. lucidum (48.86g/100g) and lowest in Amanita pantherina (13.59g/100g dw). In this study, fat content was detected to be highest in Astraeus hygrometricus (5.20g/100g dw) and lowest in P. populinus (0.113g/100g dw). In the present study, Riboflavin content was highest in G. lucidum (18.37g/100g dw) and almost absent in R. cyanoxantha (0.018g/100g dw). Ascorbic acid content was found to be highest in A. bisporus (27.15g/100g dw) and lowest in P. pulmonarius (13.38g/100g dw). All the mushrooms showed the presence of vitamin C in them as a result of which they all can have antioxidant property too. It was also found that these mushrooms contained different minerals, hence, they can be used as a good source of minerals in human diet. The highest amount of Ca was detected in P. pulmonarius. Level of K, Mg and Na was seen to be the highest in A. bisporus. Among, all the minerals potassium was found to be in highest amount. As a result of this, these mushrooms could serve as a good source of potassium and can be used against problems like, renal and myocardial damage which are caused due to potassium deficiency.

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Authors contributions

Foziya Khan has done the research under the supervision of Ramesh Chandra.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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References


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