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Physicochemical characterization and antioxidant property of powdered basidiocarp of wild *Lentinus sajor-caju*

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Abstract : *Lentinus sajor-kaju* is highly valued as nutritious food in many Asian and European countries. Recently it has shown several medicinal potentials also. The present study emphasizes physicochemical features and antioxidant properties of dried powder of wild *L. sajor-caju*. Pharmacological parameters of the dried sieved powder were investigated such as powder micrscopy, fluroscence characteristics, and organoleptic analysis. In addition, methanolic extract was prepared where extractive value was 8.2 %. Chemical profiling of the extract revealed presence of different phytochemical constituents like phenols, flavonoids, ascorbic acid, β carotene and lycopene. On the other hand, HPLC was conducted to determine phenolic fingerprint of the methanolic extract of this macrofungus. Antioxidant activity was evaluated through DPPH radical scavenging assay (EC_{50} 0.43 mg/ml) and total antioxidant capacity determining assay. Results demonstrate that the mushroom could be a promising candidate for future neutraceuticals.
Keywords: Antioxidant property, HPLC, Microscopic characters, Phytochemicals, Quality assessment.

Introduction:

Since ancient times, mushrooms have been widely accepted as delicacy with high nutritional and functional value. According to literature published, about 22,000 species of mushroom belong to the category of edible ones and among them only 10% are explored[1]. Mushrooms represent a major and largely untapped source of several bioactive metabolites with therapeutic potentials. In Chinese folk medicine, mushrooms are highly appreciated and extensively consumed by local communities[2]. To date, more than 700 species are known to possess significant therapeutic properties including antioxidant[3,4,5], antimicrobial[6,7], immune-modulatory[8,9], anticancer[10], antidiabetic[11] etc. A huge number of mushroom derived products are commercially sold in all over the world. Nowadays modern research has been mostly focused on the active constituents and therapeutic potential of mushroom. So that this unexplored organism could be successfully exploited in modern ages as neutraceuticals for the benefit of mankind.

Lentinus sajor-kaju is one such potential oyster mushroom (family Polyporaceae) distributing mostly in tropical and subtropical regions throughout the world. This mushroom is a rich source of proteins, carbohydrates, dietary fibres, especially minerals[12] and vitamins[13]. Like other oyster mushrooms, *L. sajor-kaju* can also be artificially cultivated on various agricultural and lignocelluolosic wastes[14]. Several extracts from this mushroom showed remarkable therapeutic potentiality including antioxidant[15,16], anticancer[13], anti-inflammation[17], antidiabetic[18] etc. Although data regarding pharmacological and chemical characters of dried

powdered basidiocarp of wild *L. sajor-caju* is still lacking. Therefore, this study was undertaken to investigate physico-chemical profile and antioxidant potentiality of dried powdered basidiocarp of *L. sajor-caju*.

Material and Methods:

Mushroom collection:

Fresh basidiocarps of *L. sajor-caju* were collected Baruipur regions of West Bengal during the month of July. After collection standard literature is consulted for identification of the mushroom[19]. A voucher specimen was preserved following the standard protocol[20] and kept in the Calcutta University Herbarium (CUH) with the accession number CUH AM352. Basidiocarps were then dried properly at 40 °C for overnight by a field drier to make them crispy and pulverized using an electric blender and sieved through 160 mesh and stored in an air-tight container.

Microscopic characterization of the powdered basidiocarps:

For microscopic characterization, dried sieved powder was treated with 5% KOH, Melzer's reagent, and Congo red. Then the slide was examined under Carl Zeiss AX10 Imager A1 phase contrast microscope and images were captured with a digital camera at desired magnifications.

Physico-chemical analysis:

Physico-chemical parameters of the powdered drug such as organoleptic features, fluroscence characteristics were determined. Organoloeptic features like colour, odour, taste, and nature of the powdered sample were evaluated. In addition, fluorescence characteristics of dried powder of *L. sajor-caju* was detected by treating a small pinch of dried powder with 17 different chemicals and each treated sample was observed under ordinary light and then under long and short UV light [21].

Preparation of methanol soluble extract and organoleptic characterization:

5 gm dried powder was soaked in 100 ml methanol for overnight and subsequently filtered using a Whatman no. 1 filter paper. The residue was re-extracted with 30 ml methanol and the combined filtrates were evaporated at 40 °C (Rotavapor R3 Büchi, Switzerland) to reduce the volume. The methanolic fraction was stored at -20 °C in a dark bottle until analysis, for no more than 1 month. Percentage yield and organoleptic parameters of the extract were recorded.

Phytochemical screening of methanolic extract:

Freshly prepared methanol extract was subjected to different quantititative chemical tests to find out the presence and quantity of different phytochemicals such as, phenols, flavonoids, ascorbic acid, β carotene and lycopene. The content of total phenolic compounds in extract was estimated using Folin-ciocalteu reagent [22]. Gallic acid was used as the standard. The results were expressed as µg of gallic acid equivalents per mg of dry extract. Total flavonoid content was determined using aluminium nitrate and potassium acetate [23]. Quercetin (5–20 µg/ml) was used to obtain the standard curve. The results were expressed as µg of quercetin equivalents per mg of the dry extract. β-carotene and lycopene contents were estimated using the protocol of Nagata and Yamashita [24]. Ascorbic acid content was determined by titration against 2, 6-dichlorophenol indophenol dye [25].

HPLC of methanol soluble extract

The methanolic extract was filtered through 0.2 µm filter and 20 µl of the filtrate was loaded in the HPLC system (Agilent, USA). Separation was done on an Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) using a flow rate of 0.8 ml/min at 25 °C. The mobile phase consisted of eluent A (acetonitrile) and eluent B (aqueous phosphoric acid solution, 0.1% v/v). A gradient program was used for elution: 0–2 min, 5% A; 2–5 min, 15% A; 5–10 min, 40% A; 10–15 min, 60% A; 15–18 min, 90% A. The absorbance of sample solution was measured at 280 nm[26].

Antioxidant assay

DPPH radicals scavenging activity and Total antioxidant capacity and of the methanolic extract of *L. sajor*-caju was analyzed following Mitra et al.[27].

Results and Discussion:

This study is an attempt to establish, the diagnostic characteristics of *L. sajor-caju*. All these parameters studied here are useful for identication and chemical characterization of this medicinally important macrofungus.

Microscopic study of any powdered sample is considered as one of the prerequisite steps in identification of raw materials [28] and should be executed first before any detailed methodological tests are undertaken [29]. Dried powder after passing through sieve was macerated with HNO_3 and KOH individually which showed the following micro-morphological characters (Figure 1): Hyphae dimitic (skeletal and binding), hyphal wall simple, without septation, with clamp connection, binding hyphae non-septate sometimes branched, 2.74 µm, skeletal hyphae thin walled, hyaline, without any septation 3.43 µm wide, basidiospore ovoid to ellipsoid, slightly bent thin walled, smooth (6.17-4.11×2.51-1.71) µm. Melzer's reaction showed negative result which signifies that the basidiospores are non-amyloid in nature.

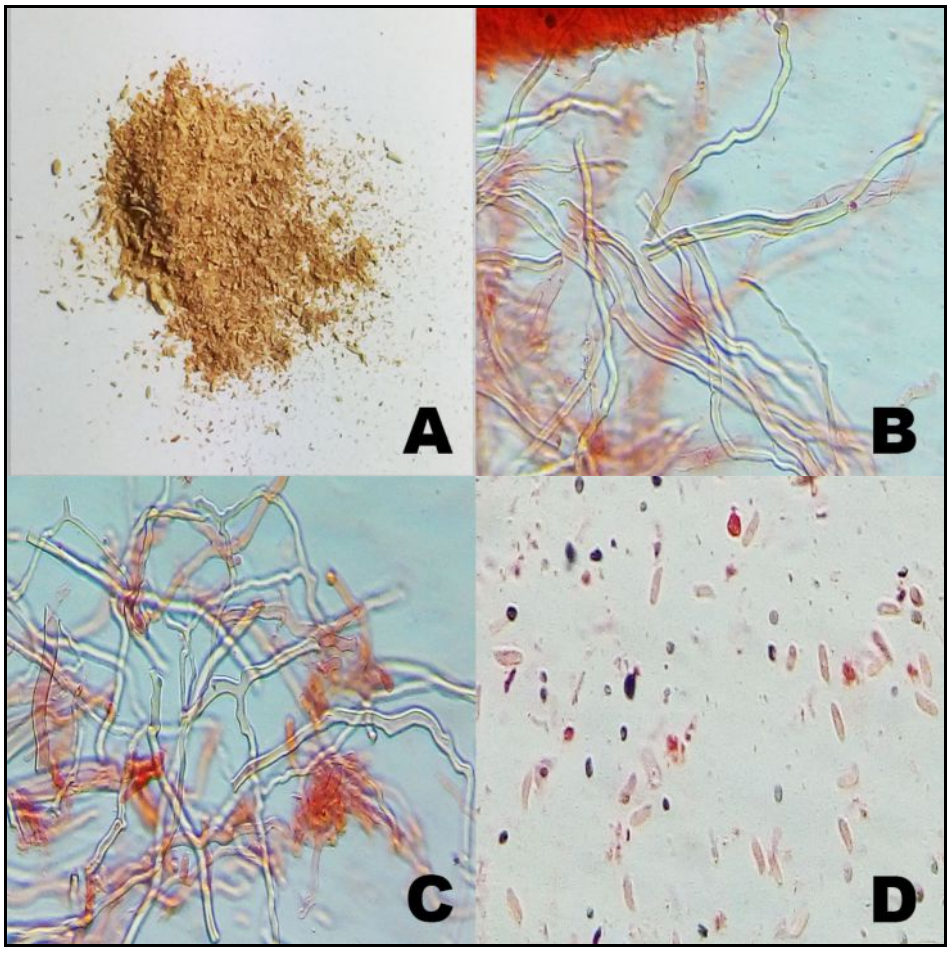

Figure 1. A) Sieved powder of *Lentinus sajor-caju*. Microscopic characterization: B-C) Hyphae D) Spores.

Organoleptic characterization ensures the most rapid and simplest technique to identify any crude drugs especially in powdered form. The organoleptic study of the sieved powder showed light brown colour, pungent odour, without any taste and granular texture. In addition to fluorescence characteristrics were also determined which revealed a remarkable variation in colours under ordinary and UV light (Table 1). These unique characters will be helpful in maintaining the quality and purity of genuine material and also provides an idea about the chemical nature.

Table 1. Fluorescence analysis of dry powder from *Lentinus sajor-caju*

Serial No.	Reagents	Visible	UV	
			Long (365 nm)	Short (254 nm)
1	Powder as such	brown	Dark brown	Light brown
2	Hager's	Light brown	Dark brown	Greenish brown
3	Mayer's	Reddish brown	Dark brown	Greenish brown
4	Dragendroff's	Brown	Dark brown	Greenish brown
5	Iodine solution	Brownish red	Dark brown	Dark greenish brown
6	1(N) HNO_3	Light brown	Blackish brown	Brownish green
7	50% HNO_3	Greenish brown	Blackish brown	Greenish brown
8	Barfoed	Greenish brown	Black	Greenish black
9	Sodium nitroprusside	Light brown	Black	Dark green
10	H2O	Brown	Dark brown	Dark green
11	$FeCl_3$	Brown	Brownish black	Brownish black
12	1(N) NaOH	Brown	Blackish brown	Brownish green
13	Acetic acid	Brown	Blackish brown	Brown
15	1(N) HCl	Brown	Blackish brown	Greenish brown
16	Methanol	Reddish brown	Blackish brown	Greenish brown
17	1(N) NaOH in Methanol	Orangish brown	Greenish black	Greenish brown

A methanolic extract was prepared from *L. sajor-caju* and the fraction was light yellow in colour with extractive value of 8.2±1.85 %. Furthermore, quantitative analysis of phytochemicals of the methanolic extract was carried out in order to evaluate pharmacological active metabolites present in this mushroom. According to the literature, phytochemicals are mainly responsible for medicinal activities of plant [30]. The extract was found to contain phenol as much as 7±0.35 µg gallic acid equivalent/mg of extract. Total flavonoid content was determined by using quercetin as standard. The extract contained flavonoid as 1.25±0.44 µg quercetin equivalent/mg of extract. Very negligible amount of β-carotene and lycopene were found such as 0.0221±0.001 µg/mg of the extract and 0.012±0.002 µg/mg of the extract respectively. Ascorbic acid was also found in minor amount (15.97±0.02 µg/mg of extract).

Table 2. HPLC chromatogram at 278 nm of methanol extract from *Lentinus sajor-caju*

Peak No.	Retention time (min)	Max. height (AU)	Area (AU)
1	6.025	144.58737	5184.41748
2	8.968	2.26467	61.89781
3	9.557	4.95568	90.90350
4	9.809	3.90503	43.21401
5	10.057	4.12704	93.87965
6	11.668	82.03270	2005.21021
7	12.161	36.67122	718.76709
8	12.768	19.36152	695.60840
9	13.895	3.81746	80.64808
10	14.447	1.75930	11.33632
11	14.743	11.07727	128.48564
12	15.281	3.79003	37.44621
13	16.149	1.64019	14.30741
14	16.577	1.41980	8.89991
15	16.766	2.16376	13.13630
16	16.916	4.08523	33.04513
17	17.186	39.32423	648.38696

In order to determine chromatographic profile of methanolic extract of *L. sajor caju*, high performance liquid chromatography (HPLC) was carried out. HPLC is one of the efficient techniques in modern prospects for preliminary determination of phytoconstituents in the sample. In the present study, Figure 2 represents HPLC chromatogram of the extract showing 17 peaks which might be of phenolic compounds. Respective areas of each peak were documented in Table 2.

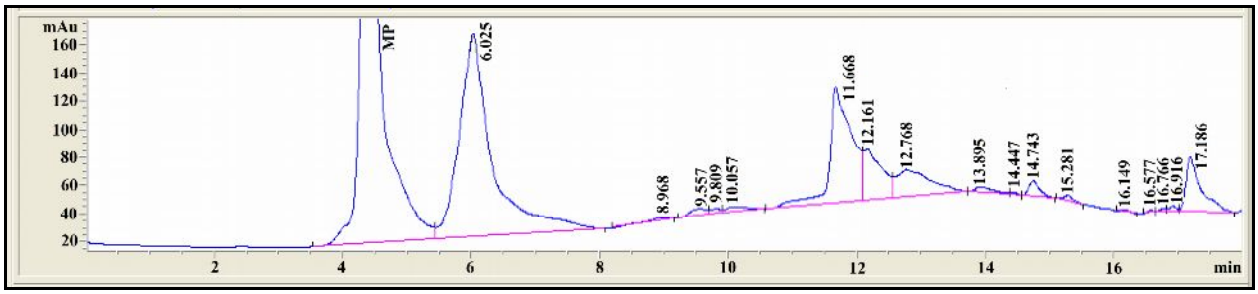

Figure 2. Enlarged HPLC chromatogram of methanol extract from *Lentinus sajor-caju* at 278 nm. (MP-Mobile phase)

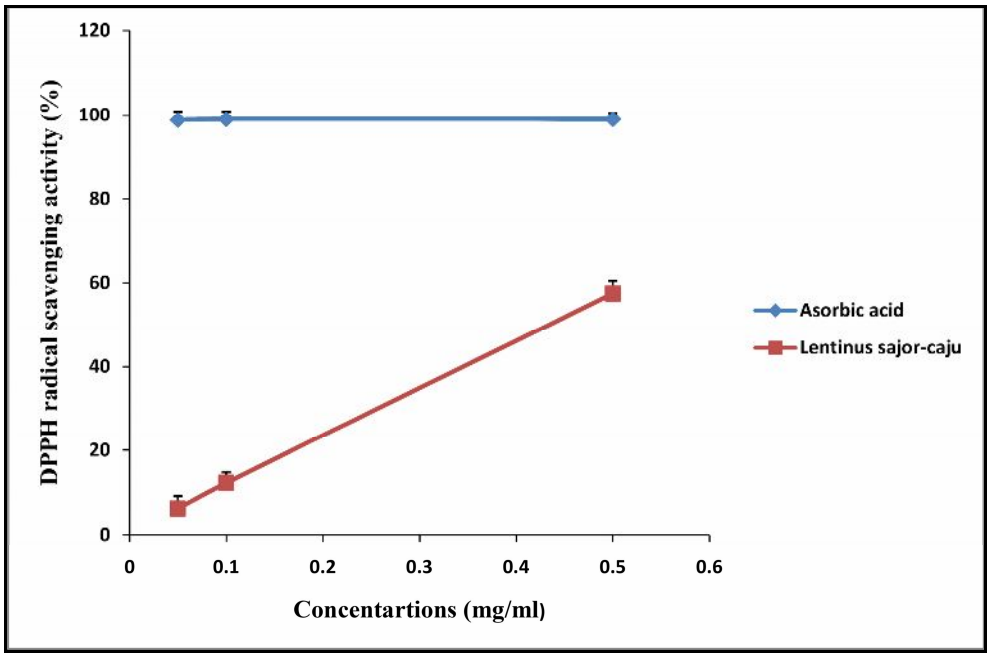

Figure 3. DPPH radical scavenging activity of methanol extract from *Lentinus sajor-caju.*

Antioxidant compounds are the major potent defense molecules that protect biological cells from free radicals induced damage. Natural source of antioxidants are mostly preferred by consumers instead of synthetic ones due to its easy availability and no toxic effects [31]. To test antioxidant potentiality of the methanolic extract, two in vitro assays are performed. DPPH radical scavenging assay is one of the most commonly use method to determine antioxidant potentiality of any compounds [32]. DPPH is a stable N_2-centered free radical which accepts an electron/hydrogen to gain stability. In methanol solution DPPH produces violet colour. Antioxidant compounds donate electrons to DPPH radicals which results in visible discolouration of methanol solution from violet to yellow [33,34]. Antioxidant screening of methanolic extract of *L. sajor caju* showed a high effective free radical scavenging activity in the DPPH assay at the rate of 6.23%, 12.28%, and 57.49% at 0.05, 0.1 and 0.5 mg/ml concentrations as represented in Figure 3. EC_{50} value was found to be at 0.43 ±0.04 mg/ml.

In addition to DPPH assay, the total antioxidant capacity of the extract was evaluated based on the formation of the phosphomolybdenum complex at acidic pH. This activity was measured spectrophotometrically at 695 nm[35] and expressed as equivalents of ascorbic acid. The extract showed 22.5 ± 1.55 µg ascorbic acid equivalent antioxidant capacity per mg of extract.

Conclusion:

From the above discussion, it is concluded that the physicochemical study is a necessity for ensuring the identity of the herbal drugs which will be also helpful for medicinal preparations. All these pharmacological parameters studied in this work will help to maintain quality and purity of powder commercially available. Besides this, antioxidant screening of this macrofungus serve it as a promising resource of antioxidant compounds.

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