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Studies On Pharmacognostic Profiles Of Three Medicinally Important Wild Edible Mushrooms

Subrata Giri[1], Gunjan Biswas[1], Subhash C. Mandal[2], Krishnendu Acharya[1]*

[1]Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata- 700 019, West Bengal, India
[2]Division of Pharmacognosy, Jadavpur University, Kolkata- 700032, West Bengal, India

Corres. author. E-mail: krish_paper@yahoo.com

Abstract: Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicines. Several mushrooms act as novel antimicrobial and chemotherapeutic agents. To evaluate correctly the identity of mushrooms, the pharmacognostic profile of three wild edible mushrooms *Lentinus squarrosulus* (Mont.) Singer ex Pegler, *Russula albonigra* (Krombh.) Fr. and *Tricholoma giganteum* Massee were evaluated. These studies include macroscopic and microscopic characteristics, powder analysis, extractive values, physical constant values, preliminary phytochemical tests, behavior of the powder material on treatment with different chemical reagents and fluorescence character of powder material under UV light. The identification of the mushroom at powdered condition is impossible. Therefore, the aggregate data from the above-mentioned experiments give a clear-cut picture at the powdered state, which ultimately help in identification and purity assessment.
Key words: Mushroom, Pharmacognosy,*Lentinus squarrosulus,Russula albonigra,Tricholoma giganteum.*

INTRODUCTION

Physical, sensory, botanical characteristics of drugs formed the main features of the study in the early period but since the latter half of nineteenth century, the emphasis was laid on crude drugs and their substitutes as adulterants because of commercial practices. Subsequently when a number of drugs were introduced in the form of powders in pharmacopoeias, a rapid progress was made in the field of microscopy. Quantitative microscopy has proved to be of great help in identification of drugs that have definite countable microscopic plant structures. Further, the use of ultra-violet light has been helpful in analytical and pharmacognostical work. Gradually the trend of study has changed and other methods have been introduced for identification and evaluation of drugs. The modern trend is, therefore, the broad scientific understanding of drugs based on biology, chemistry and co-ordination with collateral sciences which are fundamental bases of pharmacognosy. Pharmacognosists have devised various methods and procedures from time to time as and when

adulteration becomes a source of harm to public health. Besides identification of drug by macro and microscopical methods, fluorescence analysis, detection of active constituents present in drugs is also equally important.

The medicinal uses of mushrooms possess a very long tradition in Asian countries, where as their use in the Western hemisphere has been increasing slightly only since the last decade [1]. The extensive uses of mushrooms by different pharmaceutical industries coupled with the recent revival of interest in herbal medicine have led to an ever-increasing demand of them. In view of their diverse medicinal applications, and in order to ensure the quality of the raw drug, especially in the times of adulteration and substitution prevailing in the crude markets of India, the current study has been undertaken to evaluate the detailed pharmacognostic profile of the powdered fruit bodies of the samples, which will be useful to pharmaceutical industries for the authentication of their commercial samples.

MATERIALS AND METHODS

Collection

Collection of the wild edible mushrooms was carried out during the monsoon period. Fresh fruit bodies were collected from different areas of West Bengal. Information of edibility was gathered by discussion and direct interview with local people and by direct observation on the way, different mushrooms were being collected and used. The documented information was verified by cross checking with relevant literature and key informants like village elders. The following points were kept in mind during the collection of edible mushrooms. Damaged, infected and very young fruit bodies were avoided. Collections from different places or different species were kept separately. Habitat of all records were carefully noted especially forest type and substrate. Well developed fruit bodies were photographed both in the field and in the laboratory. Macroscopic characters of all the collections were thoroughly observed and noted down in the data book. Spore print colours were observed on microscopic slides in the day light, dried and stored for future reference. The specimens were dried at 40 – 50 °C in a hot-air oven and deposited at the mushroom herbarium of Calcutta University. Fresh fruit bodies were also preserved in kew spirit. Microscopic characters were studied using dried fruit bodies after reviving in 10% KOH. Then the specimens were identified according to Das and Sharma [2] and Purkayastha and Chandra [3].

Determination of extractive value

Dry powdered fruit body (5gm) was taken to determine the extractive values by using successively non polar to polar solvents starting from petroleum ether to benzene, chloroform, acetone, methanol & ethanol in Soxhlet extraction apparatus. The dried extracts were obtained after evaporation of solvent under reduced pressure [4]. The extractive value (EV) (%) was calculated in following way:

EV (%) = (Weight of dried extract / Amount of dried powder) × 100

Chemical group tests

The preliminary phytochemical studies were performed by testing different chemical groups present in different extracts of the samples [5].

Behaviour of the powdered material on treatment with different chemical reagents

The powdered materials were studied under different conditions with different chemical reagents like nitric acid, hydrochloric acid, sulphuric acid, sodium hydroxide, and colour reactions were determined [4].

Fluorescence characters of the powdered material under ultraviolet light

The fluorescence characteristics of the dried powder with respect to different chemical reagents were observed in ultraviolet light (U V light at 254 and 365 nm) [6].

RESULTS AND DISCUSSION

Macroscopic and microscopic characters

Sporophore of *L. squarrosulus* is annual, sub-infundibuliform. Pileus usually 2-7 cm wide, white to cream coloured, turning somewhat brownish with age, coriaceous and flexible when fresh, becoming stiff smooth on drying, minutely scaly; gills crowded decurrent, white to cream when young, brownish with age. Stipe central, 4.5 cm long, whitish at first, brown at maturity (Figure 1). Basidiospores hyaline, smooth, thin walled, 4.2-6.8 × 3.2-3.5 µm. Basidia clavate, tetrasterigmatic, 12.5-18.6 × 3.5-4.2 µm.

Sporophore of *R. albonigra* is annual, infundibuliform at maturity. Pileus 40-85 mm in diameter. Pileipellis viscid when wet, white to gray yellowish brown, gradually dark gray to black after bruising or maturity; margin inrolled to incurved, nonstriate, gills broadly adnate to subdecurrent, crowded, forked near the stipe, thin, white, blackening directly after bruising; short stipe 30-53 × 18-25 mm, central, cylindric to subclavate, white, quickly blackened after bruising, context white (Figure 1). Basidiospores 7.2-9.4 × 5.9-7.5 µm, subglobose to broadly ellipsoid or rarely ellipsoid. Basidia 32-65 × 7-11 µm, clavate, 2-4 spored.

Sporophore of *T. giganteum* is annual, conico-convex then expanding. Pileus 30-36 cm diameter, surface initially white, soon gray with a glaucous tint, paler towards the margin, glabrous and silky smooth but cracking on drying; margin slightly incurved, scurfy, often cracking. Lamellae emarginated, sinuate, straw yellow, ventricose, densely crowded, with lamellulae of four lengths. Stipe 15-18 × 6 cm, cylindrical, often elongate, solid, finally fistulose; surface concolorous with pileus, fibrillose-striate (Figure 1). Basidiospore 6.69-8.66 × 4.72-6.30 µm. Q=1.37-1.41, ovoid to short ellipsoid, hyaline, inamyloid, thin walled. Basidia (25.6-)27.58-30.73(-31.5) × 7.88-8.27 µm, narrowly clavate to subcylindrical, tetrasterigmatic; with basal clamp connection.

Pharmacognostic study

Certain physical characters like colour, odour and taste of the powdered material were evaluated. The extractive values were obtained after successive solvent extraction (Table 1). The primary phytochemical test was done to evaluate the presence of active constituents (Table 2). Behaviour of powdered samples on treatment with different chemical reagents and also specific fluorescent characteristics when subjected under ultraviolet light were evaluated (Table 3). The colours imparted on treatment with different reaction mixtures under different conditions of light give a characteristic simple mode of identification of powdered materials.

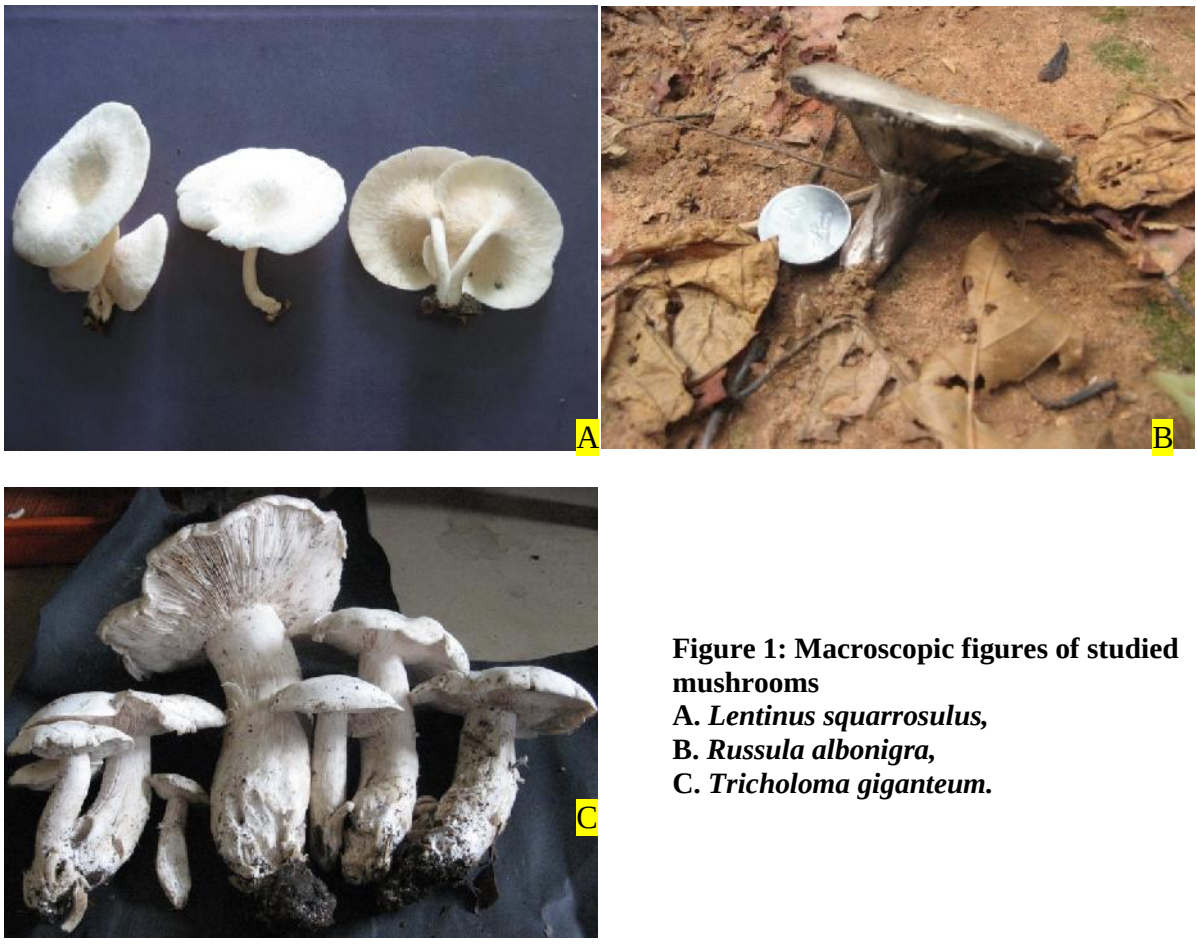

Figure 1: Macroscopic figures of studied mushrooms
A. *Lentinus squarrosulus,*
B. *Russula albonigra,*
C. *Tricholoma giganteum.*

In *Lentinus squarrosulus*, the highest percentage of extract was obtained by methanol and lowest by benzene and acetone. In case of *Russula albonigra*, the highest percentage was obtained in methanol and lowest in benzene. The lowest yield was obtained in case of acetone and maximum in methanol for *Tricholoma giganteum*. All the extracts of all the mushrooms exhibited positive presence of antioxidant flavonoids and polyphenols indicative their potent medicinal activities.

Modern scientific investigations showed that mushrooms have immense potentiality against a wide range of human ailments such as cardioprotective [7], hepatoprotective [8, 9], chemopreventive [10, 11], immunomodulatory [12], and also strong free radical scavenging activity [13, 14, 15, 16]. Several compounds extracted from mushrooms revealed antifungal and antibacterial activity [12, 17]. We have already reported potent anticancer, hepatoprotective and antioxidant activities of *Tricholoma giganteum* [18, 19, 20, 21] and *L. squarrosulus* has a potential free radical scavenging activity [22]. Besides these, in this journal we have communicated potential antimicrobial activity of the studied mushrooms (Unpublished data). The result of the former and current study may suggest that the basidiocarps of the mushrooms are a source of pharmacologically active substances having diverse therapeutic applications and they need to have a proper pharmacognostic fingerprint.

Table 1: Extractive values in the following solvents & colour of extracts of *Lentinus squarrosulus* (Mont.) Singer, *Russula albonigra* (Krombh.) Fr., *Tricholoma giganteum* Massee.

Solvents	*Lentinus squarrosulus*		*Russula albonigra*		*Tricholoma giganteum*	
	Colour of Extractives	% of Extractive values	Colour of Extractives	% of Extractive values	Colour of Extractives	% of Extractive values
Pet. ether	Colourless	0.54	Colourless	1.72	Lemon yellow	4.22
Benzene	Colourless	0.20	Colourless	0.46	Lemon yellow	1.30
Chloroform	Colourless	0.34	Colourless	0.56	Luteous	1.24
Acetone	Colourless	0.20	Colourless	0.82	Lemon yellow	0.74
Methanol	Colourless	3.34	Lemon yellow	4.92	Lemon yellow	4.70
Ethanol	Colourless	1.24	Lemon yellow	1.28	Lemon yellow	2.66

Table 2: Preliminary phytochemical tests for presence of active constituents.

Extract/constituent	*Lentinus squarrosulus* (Mont.) Singer				*Russula albonigra* (Krombh.) Fr.				*Tricholoma giganteum* Massee			
	A	S	P	F	A	S	P	F	A	S	P	F
Petroleum ether extract	-	-	+	+	-	-	+	+	-	-	+	+
Benzene extract	-	-	+	+	-	+	+	+	-	+	+	+
Chloroform extract	-	-	+	+	-	-	+	+	-	-	+	+
Acetone extract	+	+	+	+	+	+	+	+	+	+	+	+
Methanol extract	+	+	+	+	+	+	+	+	+	+	+	+
Ethanol extract	+	+	+	+	+	+	+	+	+	+	+	+

Note: + = Positive; - = Negative; A = Alkaloid; P = Polyphenols; S = Sterols; F = Flavonoids.

Table 3: Observation of florescence and standard characters of *Lentinus squrrosulus* (Mont.) Singer, *Russula albonigra* (Krombh.) Fr. and *Tricholoma giganteum* Massee powdered material under UV and normal light.

Reaction mixture (RM)	*Lentinus squrrosulus*			*Russula albonigra*			*Tricholoma giganteum*		
	Colour developed		Standard colour	Colour developed		Standard colour	Colour developed		Standard colour
	Short wave	Long wave		Short wave	Long wave		Short wave	Long wave	
RM1	Saffron	Sepia	Buff	Olivaceous black	Violaceous black	Olivaceous black	Buff	Sepia	Saffron
RM2	Yellowish green	Sepia	Saffron	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Saffron
RM3	Yellowish green	Sepia	Lemon colour	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM4	Yellowish green	Sepia	Orange	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM5	Yellowish green	Sepia	Orange	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM6	Yellowish green	Sepia	Saffron	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM7	Yellowish green	Sepia	Saffron	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM8	Yellowish green	Sepia	Orange	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange
RM9	Yellowish green	Sepia	Orange	Olivaceous black	Violaceous black	Olivaceous black	Yellowish green	Sepia	Orange

Note: RM1=Powder as such; RM2=Powder with nitrocellulose; RM3=Powder with HNO_3 in water; RM4=Powder with NaOH in water; RM5=Powder with NaOH in water dried and mounted with nitrocellulose; RM6=Powder with HCl; RM7=Powder with HCl dried and mounted with nitrocellulose; RM8=Powder treated with HNO_3 and diluted with equal volume of water; RM9=Powder treated with H_2SO_4 and diluted with equal volume of water.

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