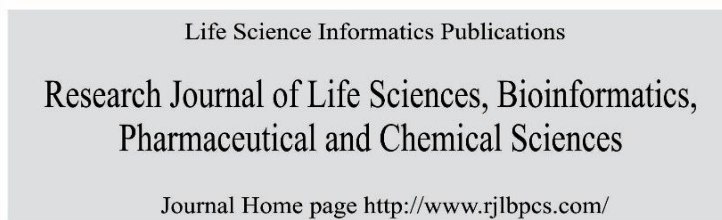

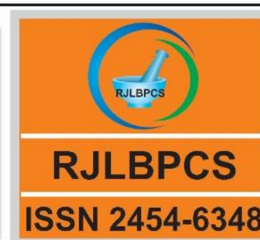

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SCREENING OF TOXICITY TEST AND EXTRACELLULAR LACCASE ENZYME OF SOME WILD EDIBLE MUSHROOMS OF TRIPURA, NORTHEAST INDIA

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ABSTRACT: In the present study, four wild edible mushrooms namely *Lentinus sajor-caju*, *Pleurotus giganteus*, *Lentinus tuber-regium* and *Panus* sp. of Tripura were screened for toxicity test by paper chromatographic method and extracellular laccase enzyme activity by using guaiacol as substrate in the medium. Toxicity test of selected wild mushrooms were showed negative result that means these wild mushrooms can be used as edible food. *L. sajor-caju*, *P. giganteus* and *Panus* sp were found to be positive activity of laccase production and *Lentinus tuber-regium* was showed negative activity of laccase. Therefore, *L. sajor-caju*, *P. giganteus* and *Panus* sp can be used as good sources of laccase for large scale production in various industrial applications.

KEYWORDS: *Lentinus sajor-caju*, *Pleurotus giganteus*, *Lentinus tuber-regium, Panus* sp. Laccase, Toxicity.

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1.INTRODUCTION

During *recent years,* in various industries enzymes have acquired enormous importance; among extracellular enzymes laccase is one of them widely present in nature and in enzymatic systems laccase is the oldest and the most studied enzyme [1]. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.) is an extracellular, multicopper bearing lignolytic enzyme; by a radical catalyzed reaction mechanism laccase catalyzes molecular oxygen to oxidize various aromatic and

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nonaromatic compounds [2-4]. Currently much attention on laccase because of its diverse applications such as detoxification of environmental pollutants, paper processing, prevention of wine discoloration and production of chemicals from lignin; several dye structures degrade bylaccase [5] transform toxic compounds into safer compounds and can be useful to control environmental pollution [6]. In oxidative methods for the decomposition of azo dyes laccase are also useful [7]. For large scale application of laccase, the present effort is to search for highly proficient laccase producing fungi with secretion of plentiful amounts of laccase and to reduce the cost of production. In higher plants, fungi and bacteria laccase is most widely distributed [8,9]. Extracellulary laccase is secreted out in the medium by various fungi during the secondary metabolism but all fungal species of Zygomycetes and Chytridiomycetes do not secretelaccase [10]. Fungi belong to Ascomycetes, Deuteromycetes as well as Basidiomycetes are known producers of laccase [11,12]. Mushrooms are known as macro-fungus with a distinctive fruiting body enough to be seen with the naked eyes and to be picked by hand; it can be either epigeous or hypogeous [13]. Out of 5000 different species of known mushrooms, at least 1220 are reported to be edible which could be employed for foods and medicines [14].There have been increasing interests in mushrooms utilization worldwide. Mushrooms are a treasure trove of several enzymes with biotechnological significance and industrial potential .In the present study, we focused on toxicity test of some wild edible mushrooms of Tripura and screening of their laccase enzyme activity.

2. MATERIALS AND METHODS

2.1.Collection of wild edible mushrooms

Wild edible mushrooms were collected from different markets of Tripura, North-East India. Morphometric study of collected wild edible mushrooms were done with photographic evidences and comparing the morphological characters with the work of Pegler in 1977 [15], Purkayastha and Chandra in1985 [16]. Lab collection number was given for each collected sample.

2.2.Isolation of pure mycelial culture of selected mushrooms

Tissue culture of mushrooms was done on either malt extract agar (MEA) or potato dextrose agar (PDA) by tissue taken from junction point of stipe and pileus of fresh fruit body after surface sterilization of the fruit bodies with 0.1% $HgCl_2$. Slants were incubated for 5 to 7 days in a closed aseptic chamber. The mycelium collected from the growing edge was transferred into fresh media (MEA and PDA) and incubated further for 5 to 7 days at 30^0C (± 5^0C). Such reinoculation repeated 2 to 3 times to get pure isolate. Pure mycelial cultures were used for molecular identification.

2.3.Toxicity test of selected wild edible mushrooms

Toxicity test of selected wild mushrooms were done by the paper chromatographic method [17]. The fresh mycelial mat (15days old) of wild edible mushrooms were separated from the adherent medium by filtration followed by several washing with distilled water. A sample of 2 g fresh mycelia mat for each test fungus was minced separately and made the volume up to 100 ml with methanol

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in a beaker. The extraction will be done by heating the methanol with mycelial fragments to boiling in water bath and was kept it for 2 minutes after removing from water bath. During extraction the content of the beaker were stirred and the tissue were pressed on the side wall of the beaker with a stirring rod occasionally. The mat were separated by centrifugation. The mat was discarded and the methanol extract was evaporated to dryness on a rotary evaporator. The residue were dissolved in one ml of methanol. The concentrated methanol solution were used to run a chromatogram, using a strip of chromatographic paper. A mixture of methyl ethyl ketone, acetone, water and butanol (20:6:5:1 v/v) were used as solvent. After 40 minutes the strip was removed and hung by the top for drying. It was then sprayed with solution of 1.0% cinamaldehyde in methanol allowed to dry and than suspended in a stopper glass container above concentrated hydrochloric acid for fume treatment. The appearance of one or more violet or blue coloured spots on the papers indicate the presence of amanitin and phallodin toxins.

2.4.Screening of laccase enzyme activity

Screening test of laccase activity of selected mushrooms *P. giganteus, L. tuber-regium* and *Panus* sp were done by the method of Coll et al. 1993 [18]. Screening test was done on plates containing following media composition (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 agar (pH-6) supplemented with 0.02% guaiacol. Five mm of 7 days old mycelial plugs of selected mushrooms were inoculated into prepared plates and the plates were incubated at 30°C for 7 days. Control (Negative activity) was maintained using the plate without inoculate mycelial plug. Laccase activity was observed on plates containing 0.02% guaiacol; laccase enzyme catalyzes the oxidative polymerization of guaiacol to form reddish brown zones around the mycelial culture in the medium.

3. RESULTS AND DISCUSSION

3.1.Selection of mushrooms

In the present study some wild edible mushrooms were collected from different markets of Tripura, Northeast India and the accession number obtained from National Center for Biotechnology Information (NCBI) of selected mushrooms were shown in Table 1. Fruit bodies and mycelial culture of selected wild edible mushrooms *Lentinus sajor-caju*, *Pleurotus giganteus*, *Lentinus tuber-regium* and *Panus* sp. were shown in fig. 1.

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Table 1. Selected wild edible mushrooms collected from different markets of Tripura with NCBI accession number

Sl no.	Lab collection number	Name of wild edible mushroom	Family	Name of markets mushrooms were collected	NCBI accession no.
1	MCCT186	*Lentinus sajor-caju*	Polyporaceae	Lake chowmuhani, Bishramganj and Teliamura	MG198766
2	MCCT 187	*Panus* sp	Polyporaceae	Lake chowmuhani and Mungiakami	MG279699
3	MCCT 189	*Pleurotus giganteus*	Pleurotaceae	Lake chowmuhani and mungiakami	MG198772
4	MCCT 190	*Lentinus tuber-regium*	Polyporaceae	Lake chowmuhani, Bishramganj, Jirania and Champak nagar	MG198774

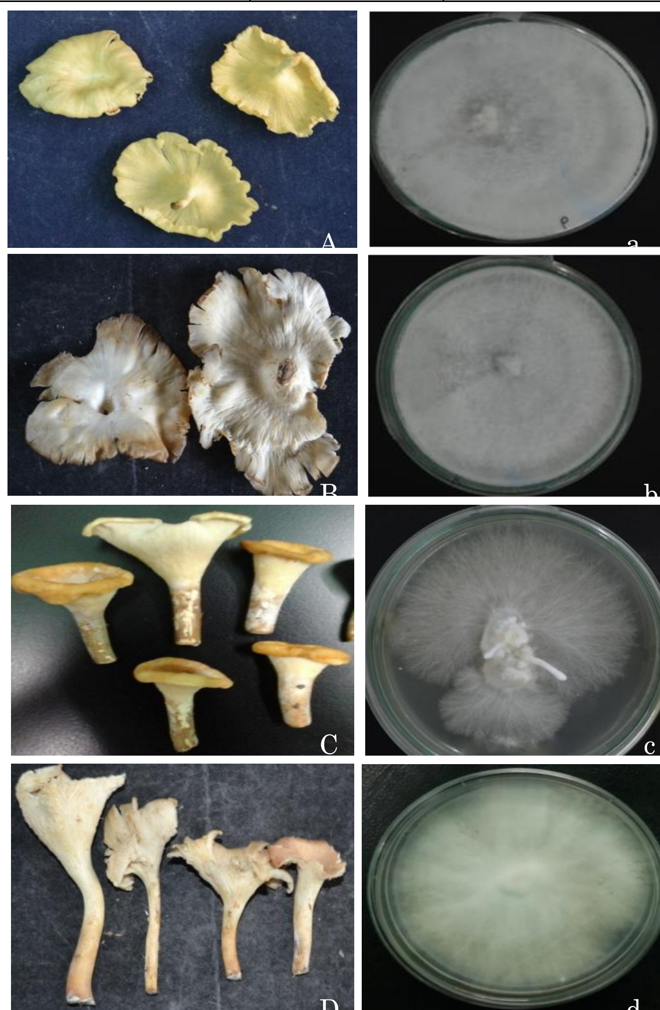

Figure 1. Fruit bodies and mycelial culture of selected wild edible mushrooms ; A-a *Lentinus sajor-caju*, B-b *Pleurotus giganteus*, C- c *Lentinus tuber-regium* and D-d *Panus* sp.

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3.2. Toxicity test

Toxicity test of selected wild mushrooms were shown in fig 2. According to Block et al. in 1955 [17] poisonous or toxic mushroom shows blue or violet color spot in the chromatographic strips. In the present study there was no blue or violet color spots appeared in the chromatographic strips. This observations indicated absence of any amanitin and phalloidin toxins in the test wild mushrooms and these mushrooms can be used as edible.

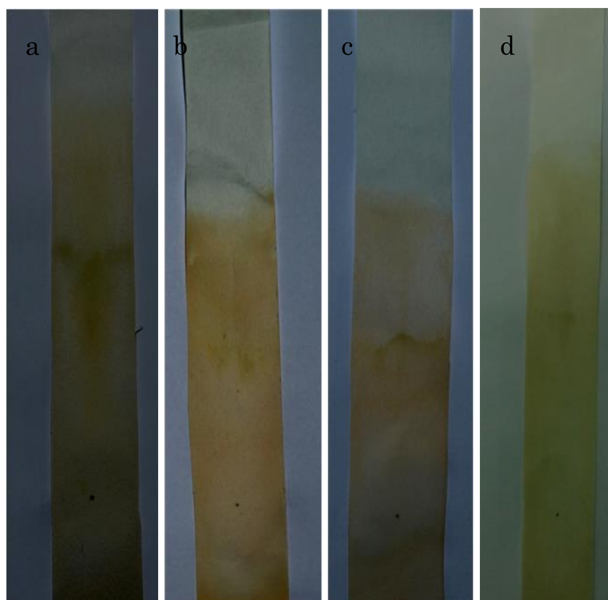

Figure 2. Toxicity test of selected wild mushrooms a) *Lentinus sajor-caju*,b)*Pleurotus giganteus*, c) *Lentinus tuber-regium* and d) *Panus* sp.

3.3. Screening test of laccase activity

Four wild edible mushrooms namely, *L. sajor-caju*, *P. giganteus*, *L. tuber-regium* and *Panus* sp. were screened for visual confirmation for the presence or secretion of laccase enzyme by using guaiacol in the media; guaiacol is one of the substrate of laccase enzyme and the degradation product of guaiacol is reddish brown in colour. Various authors reported that the laccase enzyme catalyzes the oxidative polymerization of guaiacol to form reddish brown zones in the medium [19-22]. In the present investigation, we used the reddish brown color zone around mycelial culture in the medium to indicate the laccase production by the mushroom and the diameter of the colored circle to determined the ability of mushroom for lacasse production. All plates inoculated with mycelial cultures of *L. sajor-caju*, *P. giganteus* and *Panus* sp. were showed a reddish brown color except for the plate inoculated with *L. tuber-regium* (fig. 3).

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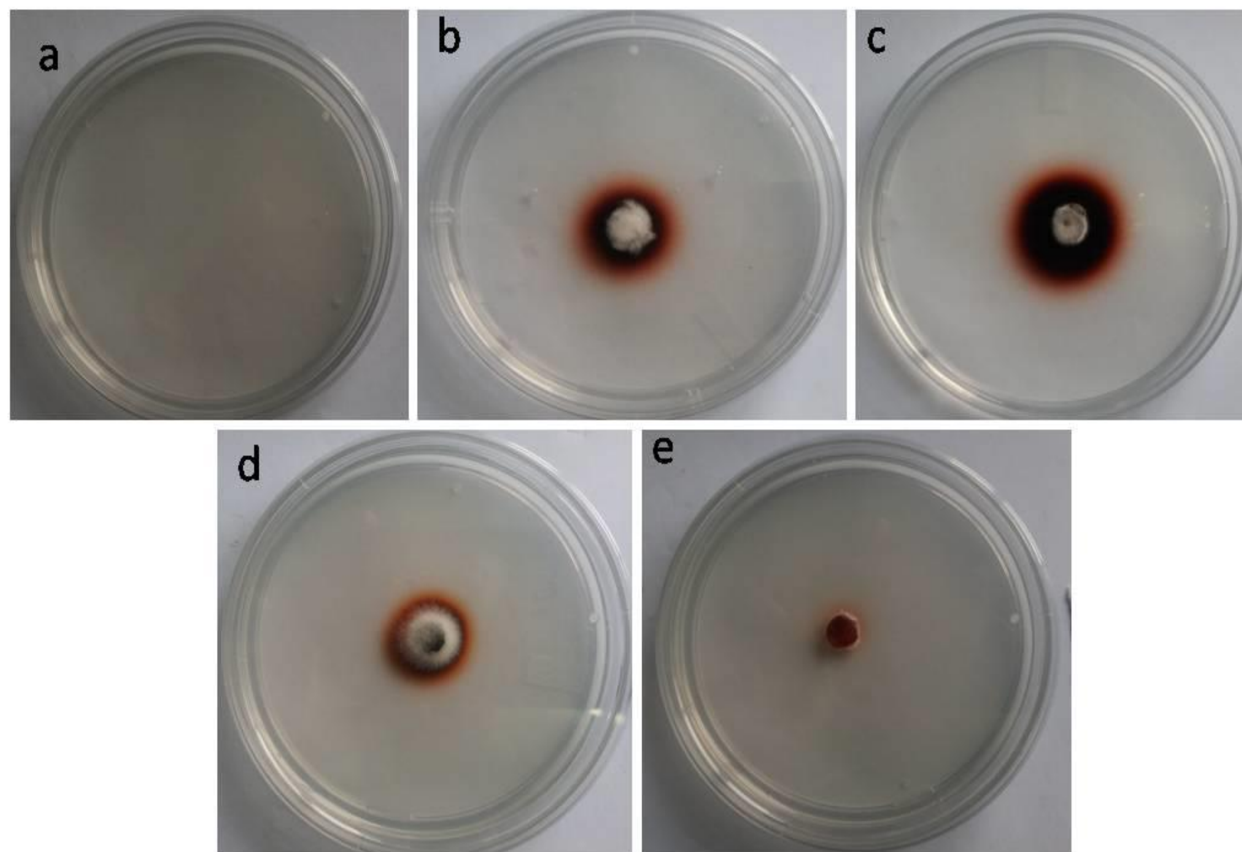

Figure 3. Screening of extracellular laccase activity a) Control (without mycelial plug), b) *Lentinus sajor-caju, c) Panus* sp, d) *Pleurotus giganteus*and *e) Lentinus tuber-regium.*

Results of screening test of laccase activity were recorded in the table 2. *Panus* sp was showed large reddish brown zone and revealed that strong lacasse activity, *L. sajor-caju* and *P. Giganteus*were showed moderate activity and *L. tuber-regium*was showed negative activity of laccase enzyme.

Table 2: Results of screening test of laccase activity of selected wild edible mushrooms

Name of mushroom	**Laccase activity**
Lentinus sajor-caju	++
Panus sp	+++
Pleurotus giganteus	++
Lentinus tuber-regium	-

+++ stands for strong laccase activity

++ stands for moderate laccase activity

- stands for negative activity

4. CONCLUSION

In the present study, it is concluded that wild edible mushrooms *L. sajor-caju*, *P. giganteus*, *L. tuber-regium* and *Panus* sp. of Tripura can be used as edible food. *L. sajor-caju*, *P. giganteus* and *Panus* sp can be used as good source for large scale laccase production which is having various industrial

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applications.

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CONFLICT OF INTEREST:

The authors have no conflict of interest.

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