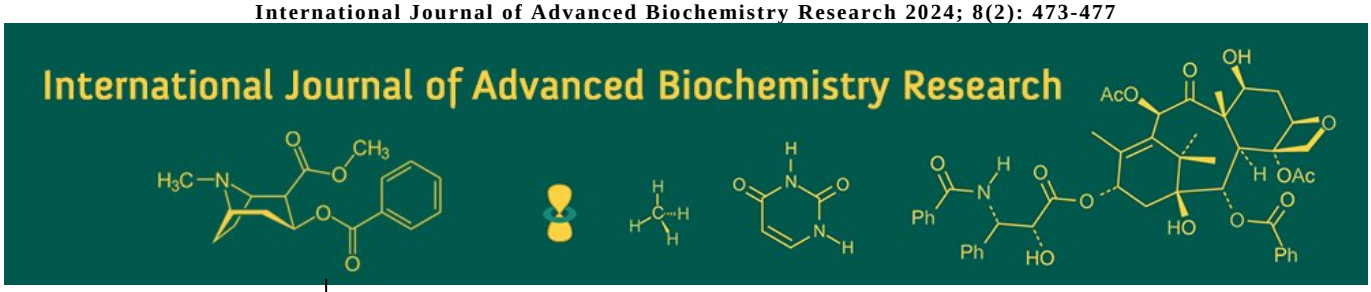

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Estimation of lovastatin from *Pleurotus species* by thin layer chromatography and spectrophotometer

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Abstract
In the global market for oyster mushrooms (*Pleurotus* species) play a significant role. In many countries, several species are grown for food on both a large and small commercial scale. In this experiment, the determination of lovastatin in *Pleurotus* spp. were screened including strains namely, *P. djamor*, *P. florida*, *P. ostreatus*, and *P. sajor-caju*. Lovastatin was produced in solid-state fermentation as rice straw and PD broth. We observed that the presence of lovastatin at Rf value is 0.94 to 0.97 by thin layer chromatography (TLC) from different *Pleurotus* species and found that higher lovastatin concentration obtained from *P. ostreatus* at mycelial stage followed by *P. florida* at pinhead stage, the concentration of lovastatin is (545±21.3mcg/g) and (485±21.3 mcg/g) from the sample by using UV spectrophotometer at 513 nm.

Keywords: Lovastatin, *Pleurotus* spp., thin layer chromatography, spectrophotometer, solid state fermentation etc.

Introduction
A diverse group of the most widely grown mushrooms, presently oyster on mushroom contribute 19% respectively in terms of total mushroom production in the world [1]. It is a lingo-cellulolytic fungus of basidiomycetes, they are grown on diverse agricultural, forestry and industrial waste products and by using these wastes, they reduce the risks to the environment and human health [2]. Also, the fruiting bodies of oyster mushrooms are a valuable source of lovastatin, a member of the class of compounds is known as statins. Statins, a precise effective competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting reduction of HMG-CoA to mevalonate in mevalonate pathway of cholesterol biosynthesis, has revolutionized the treatment of hypercholesterolemia [3]. Lovastatin ($C_{24}H_{36}O_5$), formerly known as Mevinolin, Monacolin K and Mevacor, was the first statin to be approved by the US Food and Drug Administration (USFDA) as a potent hypercholesterolemia medication [4].
Lovastatin is a secondary metabolite produced during the secondary phase of fungal growth exists as open β-hydroxyl form and the synthetic lactone form [5]. It was first isolated from *Aspergillus terreus* in 1978 and introduced into the medical field by Merck in 1987 [6]. Many researchers were interested in finding a better alternative source of fungi that could produce lovastatin, which led to the identification of fungi including *Monascus ruber*, *Aspergillus flavus*, *Pleurotus* species [7]. Lovastatin is formed of a six- part lactone ring with a hydroxyl group and part-hydrogenated naphthalene with a hydroxyl substituent (Fig. 1), which is esterified with a 2-methylbutyric acid residue. While lactone, which is prominent in living things, is insoluble in water, the active form of lovastatin, hydroxy acid is water soluble [8].
In the current investigation, lovastatin was produced using solid-state fermentation. There are several reasons and a vital aspect is higher production. For instance, [9] stated that is highly advantageous, the reasons such as usage of wide range of agricultural wastes and thereby reduced overall cost better yield and stability of the product.

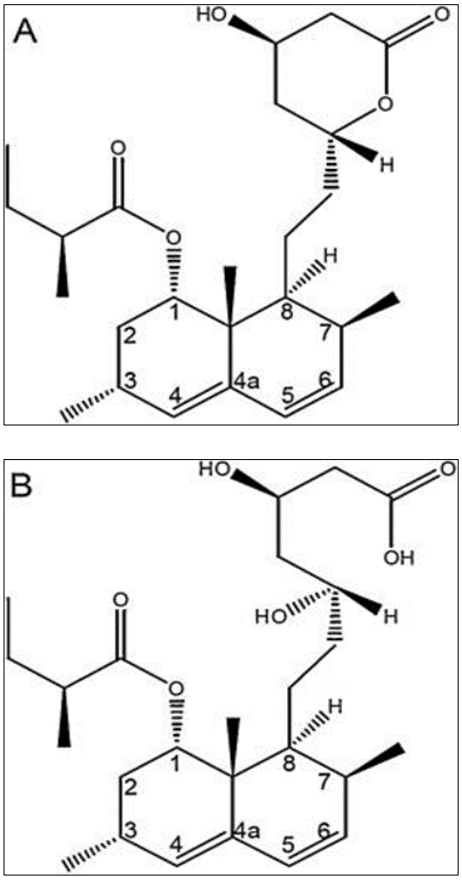

Fig 1: Two of lovastatin (A) hydroxyl and (B) lactone form

Material and Methods

Culture preparation

Oyster mushroom of four species was examined in this research. Those are *P. djamor*, *P. florida*, *P. ostreatus and P. sajor-caju*, were collected from the Plant Pathology Department, IGKV, Raipur, (C.G.). Culture was maintained on Potato dextrose agar (PDA) media composition having potato starch 20 g, dextrose 20 g, agar 2 g and distilled water to make final volume 1 liter. The media was sterilized at 121 °C for 30 min. at 15 psi, then it was cooled to 40 °C and stored at 4 °C [10]. Three stages were used for this purpose i.e., pinhead, mycelial and fruiting bodies. Only mycelia growth condition was differed from them was PD broth.

Solid- state substrate preparation

Paddy straw was used as the substrate for solid state fermentation, which was purchased from local merchants. The substrates were soaked in water overnight to produce a moisture level of 65% then the paddy straw exposed to sunlight by spreading it on a clean and disinfected platform, ensuring that the moisture level of the substrate remained around 60%. Per bag of substrates, three to four layers of spawning have been applied. The weight of the bags was then measured using an electronic weighing balance and incubated there for 21 days at 28 °C then growth was carefully observed.

Extraction of lovastatin

The lovastatin extraction process was based on previous work with some modification [11] dried sample of the fruiting body, pinhead and mycelia of *Pleurotus* species at 80 °C for 24 hours, crushed and powdered. 1 gram of dry powder of substrate was dissolved in 10 mL of ethyl acetate and kept in a shaker for 2 hours at 180 rpm. After 2 hours it was filtered with Whatman No.1 filter paper. 1 mL of 1% trifluoroacetic acid was added. The mixture was then concentrated for 30 minutes (lactonization of hydroxyl acid form of lovastatin). 1 ml of acetonitrile was added, the extract was further used in TLC and spectrophotometer analysis.

Detection of Lovastatin by TLC

Procedure given by [12] for TLC analysis was slightly changed. Using a TLC plate made of silica gel (20 X 20 cm), to make the silica plate, take 5 gm silica gel G and 10.5 ml distill water. Now mix it to make slurry. After making slurry, it transferred into clean beaker this mixture is spread as thick slurry on an unreactive carrier glass plate. The resultant sheet was heated in an oven set at 80°C for a 1 hours for the activation of glass plate. The thickness of the absorbent layer was typically around 0.1- 0.25mm. Drop from the extract was applied into the standard silica gel. Dichloromethane and ethyl acetate (70:30 v/v) were combined as mobile phase. The material was examined using iodine vapor. Rf values was calculated and compared with standard lovastatin (Tokyo Chemical Industry Co., Ltd).

Preparation of standard solution for lovastatin.

The lovastatin stock solution was prepared by dissolving at accurate weighted amount (5mg) of lovastatin standard (1000µg/ml) in 5ml of 75% ethanol and stored at brown glass bottle. This stock solution was stored at 4 °C until preparation of working solution by adding a required volume of 75% ethanol. Working solution of different concentration i.e., 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 then 1000 µl of 75% ethanol was added in each tube [13].

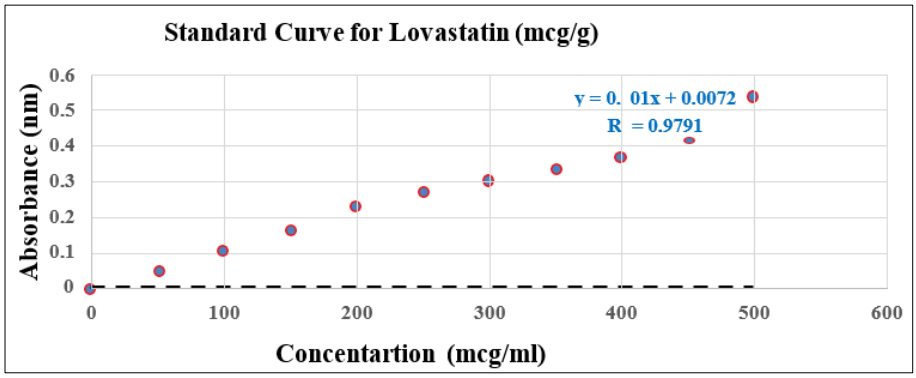

Fig 2: UV spectrophotometer based plotted the standard curve of lovastatin

Determination of lovastatin from the samples

Extracts obtained after the drying and dissolved sample of ethyl acetate and kept in a shaker for 2 hours at 180 rpm. Then filtered (Whatman No.1 filter paper) and add of 1% trifluoroacetic acid and concentrate. Add acetonitrile and then, analyzed. Prepared sample were analyzed quantitatively at 513 nm [14], subsequently lovastatin was estimated using standard lovastatin in UV spectrophotometer.

Statistical analysis

Results & Discussion

Spawn of all the four types were subjected to cultivation method such as SSF and the mycelium, pinhead and fruiting body of oyster mushroom are dried and powered before lovastatin extraction process. The different *Pleurotus* species showed a Rf value of for mycelium 0.95, 0.95, 0.96, 0.95 for pinhead 0.95, 0.95, 0.96, 0.95 and for fruiting body there is 0.95, 0.97, 0.96 and 0.96 by TLC (fig.1) and their Rf value was compared against the standard lovastatin Rf value of 0.96, 0.96, 0.97. The Rf value of *Pleurotus* extracts were similar to the standard lovastatin, which confirms that the *Pleurotus* species as a lovastatin compound. Presence of lovastatin was confirmed by thin layer chromatography in this method. The distances travelled by each spot in baseline and Rf values were calculated by comparing the standard Rf values for the chosen mobile phase, the lovastatin present in the samples was identified by [15, 16]. Plate was kept in photo-documentation for each TLC run, lovastatin standard was applied for Rf comparison. This method found to be specific for lovastatin determination (Rf value=0.96) in presence of other excipients in the standard formulations. The specificity was confirmed by the standard lovastatin on TLC. The correlation coefficient (r=0.9791) (Y= 0.001x + 0.0072) and other method validation parameters are given in next paragraph [17].

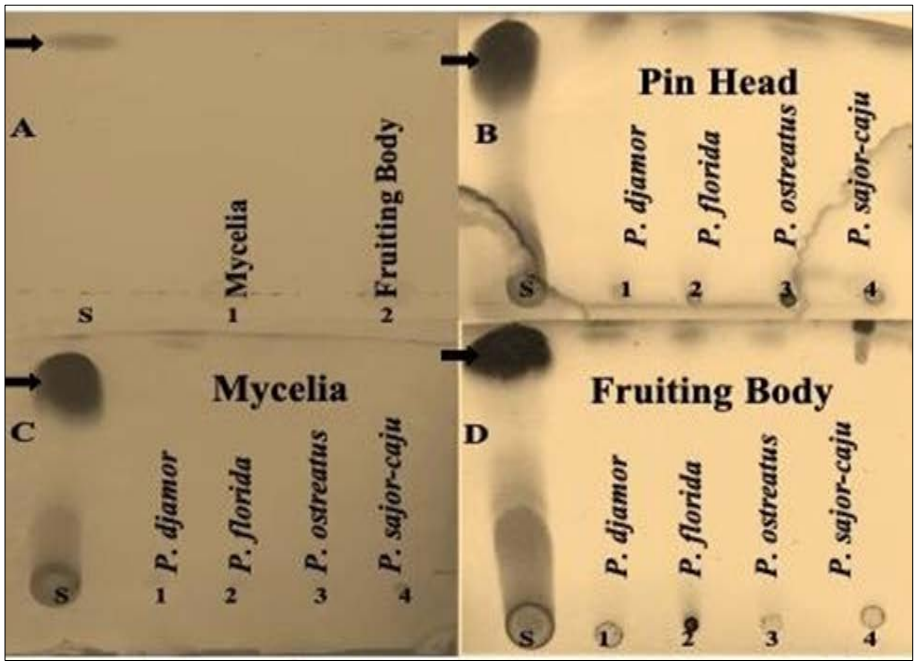

Fig 3: Chromatographic representation of TLC (A) Standard S- standard, 1- Mycelia and 2. fruiting body of *P. florida.* at pinhead stage B, mycelial C, and D Fruiting body stage and *P. djamor 1, P. florida 2, P. ostreatus 3 and P. sajorcaju* 4, arrow indicates spot of lovastatin.

Table 1: Rf value of standard lovastatin and four different samples from TLC

S. No.	Developmental stage	Standard	*P. djamor*	*P. florida*	*P. ostreatus*	*P. sajorcaju*
1.	Mycelium	0.96	0.95	0.95	0.96	0.95
2.	Pinhead	0.96	0.95	0.95	0.96	0.95
3.	Fruiting body	0.97	0.95	0.97	0.96	0.96

The determination of lovastatin content from different species of oyster mushroom like *P. djamor*, *P. florida*, *P. ostrestus*, *P. sajor-caju* under different culture condition like solid state fermentation. The results of scanning the UV spectra of lovastatin standard and *Pleurotus* different species and their ethyl acetate extracted from the fermentation broth in 75% ethanol. The concentration of lovastatin was calculated spectrophotometrically (two replication). The sample and the standard exhibited a peak at 513 nm in the spectrophotometer absorption. From the results, it was deduced that four *Pleurotus* species and their stages showed positive result for lovastatin production. Chen *et al*., 2012 reported that the fruiting bodies of *P. ostreatus* from Japan, Korea, and Taiwan gave 606.5, 165.3, and 216.4mcg/g lovastatin yield, respectively [18]. Our results also confirmed that mycelia of *P. ostreatus* (545±21.3mcg/g), pin head of *P. florida* (485±21.33mcg/g) and myceilia of *P. sajor-caju* (480±14.23mcg/g) yielded the maximum amount of lovastatin, while, fruiting body of *P. djamor* (374±7.13mcg/g), *P. ostreatus* (379±14.23mcg/g) *and P. sajor-caju* (394±7.13mcg/g) minimum production of lovastatin. And overall production of lovastatin was obtained from the dried samples of *Pleurotus* species (449±16.6mcg/g).

Table 2: Data representation of spectrophotometric analysis of four different *Pleurotus species* dried samples at three different stages

Stages	Name of species	Mean	SD	SE	RSD
Pin Head	*P. djamor*	475±7.1	7.1	5.0338	1.5
	P. florida	485±21.3	21.3	15.102	4.39
	P. ostreatus	*460±0*	0	0	0
	P. sajor caju	*440±14.2*	14.2	10.068	3.23
	P. djamor	*374±7.1*	7.1	5.0338	1.9
FruitingBody	*P. florida*	*440±42.6*	42.6	30.203	9.69
	P. ostreatus	*379±14.2*	14.2	10.068	3.74
	P. sajor caju	*394±7.1*	7.1	5.0338	1.8
	P. djamor	*460±28.4*	28.4	20.135	6.18
Mycelia	*P. florida*	*455±21.3*	21.3	15.102	4.68
	P. ostreatus	*545±21.3**	21.3	15.102	3.91
	P. sajor caju	*480±14.2*	14.2	10.068	2.96
		449±16.6	16.6	11.746	3.69
			Mean		449±16.6
			N		12
			SD		16.6
			SE		11.7
			RSD		3.69

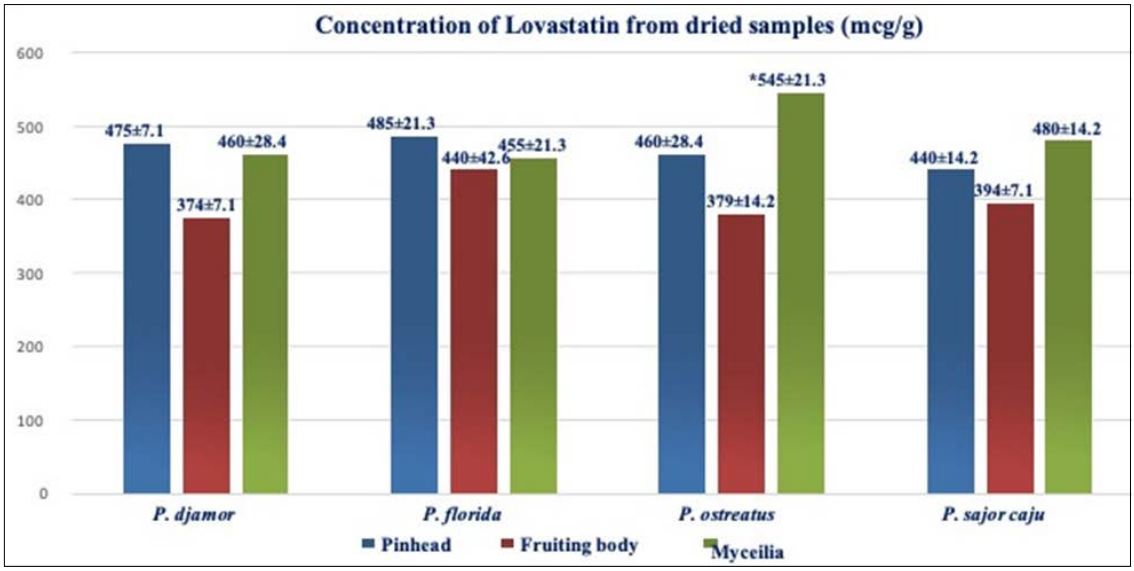

Fig. 4: Lovastatin concentration in different type of oyster mushroom, at three stag, Pin head, Fruiting body and Mycelia of *P. djamor*, *P. florida*, *P ostreatus* and *P. sajor-caju*.

Conclusion

Pleurotus spp. is an important filamentous fungus, which has been applied in the Pharma industrial production of the bio-based compound and the lipid lowering drug lovastatin. Different extracts from four edible mushroom species were analyzed quantitatively and qualitatively using spectrophotometer and thin layer chromatography to determine the presence and concentration of lovastatin in SSF. TLC revealed that *Pleurotus sp*. produce lovastatin in the present experimental conditions and also reported a higher amount of lovastatin produced by *P. ostreatus* followed by *P. florida* compared to other *Pleurotus* spp.

Conflicts of Interest

The author declares no conflicts of interest.

Acknowledgement

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