KAVAKA 57 : 33-37 (2021) doi: 10.36460/Kavaka/57/2021/33-37

Quantification of lovastatin in two wild species of oyster mushrooms from India

Amita[1*], Balwinder Kaur[1] and N.S. Atri[2]
[1]*Department of Botany, Sh. LBS, Arya Mahila College, Barnala (Punjab), India*
[2]*Department of Botany, Punjabi University, Patiala-147002, Punjab, India*
**Corresponding author Email: amitarikhi@gmail.com*

(Submitted on August 31, 2021; Accepted on December 08, 2021)

ABSTRACT

Lovastatin ($C_{24}H_{36}O_5$) is one of the potentially used drugs for the reduction of blood cholesterol levels. It is reported to competitively inhibit the 3-Hydroxy-3-methyl glutaryl Co A (HMG Co A) reductase enzyme which acts as a rate limiting step in the cholesterol biosynthesis. Lovastatin is produced as secondary metabolite by various fungi including *Pleurotus* species. These mushrooms are gaining more importance as compared to other medicinal mushrooms in terms of health promoters and as environmental restorers resulting in upsurge in their R and D activities during the past decades. The present study pertains to the quantification of lovastatin from two indigenous strains of *Pleurotus cystidiosus* O.K. Mill and *P. sapidus* Quél. For this purpose 15 days old culture of both the species growing in liquid yeast glucose medium was used. The study with spectrum analysis and High performance liquid chromatography techniques confirmed the synthesis of lovastatin by two fungi.

Keywords: Oyster mushroom, Indigenous strains, Lovastatin, Cholesterol, Nutraceutical importance.

INTRODUCTION

According to World Health Organization (WHO), an estimated 17.3 million lives are reported to have been lost in the year 2008 and an expected number of 23.6 million people are likely to lose their life by the year 2030, due to cardiovascular diseases (WHO, 2011). One of the major factors associated with cardiovascular disease is hypercholesterolemia (the presence of high levels >200 mg/dl) of cholesterol in the blood (Kelly *et al.,* 2015). One of the nutraceutically important compounds produced by number of fungi including *Pleurotus* species is lovastatin (Samiee *et al.,* 2003). It is an approved drug by the Food and Drug Administration (FDA) and United States Food and Drug Agency which is widely used in the treatment of dys-lipidemia (Vagelos, 1991; Vagelos and Galambos, 2004). Lovastatin is reported to inhibit the synthesis of cholesterol in blood by the competitive inhibition of enzyme, 3-hydroxy-3-methyl glutaryl (Csaba *et al.,* 2016). An enzyme HMG Co-A reductase is reported to play a significant role for the conversion of enzyme HMG Co-A to mevalonate (Endo, 1992; Bobek *et al.,* 1997). HMG Co-A reductase in association with higher concentration of lovastatin in comparison to HMG Co-A result in the blockade of production of mevalonate (Raghunath *et al.,* 2012). Hence it inhibits the biosynthesis of cholesterol through the mechanism of competitive inhibition (Tobert, 2003).

Lovastatin is reported to exert multidirectional effect despite of their specific mechanism of action. It helps in stabilization of atherosclerotic plaque, acts as anti-coagulant, anti-inflammatory and immune-modulatory stimulation substance (Kavalipati *et al.,* 2015). According to Bearden *et al.* (2016) lovastatin is reported to help in lessening of Alzheimer's disease levels in animal's cell culture. Ade Kallas *et al.* (2016) and Eckert *et al.* (2005) reported the antitumor capability of lovastatin. This compound is reported to suppress the function of genes involved in cell division, decrease the activity of B and D_1 cyclins and increase the activity of cell cycle inhibitors because of which it finds application during chemo and radiotherapies in cancer patients (Zeigler *et al.,* 2016; Matusewicz *et al.,* 2015; Bhargavi *et al.,* 2016). There are number of reports that revealed the pleiotropic effect of lovastatin on various diseases including osteoporosis, non alcoholic fatty livers, neuro-degeneration and rheumatoid arthritis (Butterfield *et al.,* 2011; Das *et al.,* 2015; Wang *et al.,* 2016; Doumas *et al.,* 2018). Radha and Lakshmanan (2013) also reported the counteractive action of lovastatin in kidney treatment.

The main objective of present study was aimed at the qualitative and quantitative estimation of lovastatin from the local indigenous strains of two edible oyster mushrooms, namely *P. cystidiosus* O.K. Mill and *P. sapidus* Quél. using UV spectrophotometeric analysis and high performance liquid chromatography techniques.

MATERIALS AND METHODS

Fungal Strains

The lovastatin producing strains used presently are our own lab cultures of *Pleurotus cystidiosus* and *P. sapidus*. Both the specimens were collected from the wild and raised into pure cultures through tissue culture technique. The stock cultures of both the strains were maintained at -4°C in malt extract agar (MEA) medium. The cultures of *P. sapidus* have been deposited at IMTECH Chandigarh under MTCC No. 10943 and ICAR-Directorate of Mushroom Research, Chambaghat, Solan under accession number DMRP-393 while that of *P. cystidiosus* have been deposited in the ICAR-DMR Culture Bank under accession number DMRP-394. Before use in experiments, the cultures were propagated twice in MEA medium at 28±1°C for 10 days.

Standards

Pure lovastatin standard and all HPLC grade chemicals were purchased from HiMedia Laboratories Pvt. Ltd. India. Calibration chart was prepared using standard lovastatin by dissolving it with 1 mL ethanol and made up to a volume of 5 mL using distilled water.

Medium and culture conditions

For flask culture, 10 days old cultures of both the strains were inoculated into 150 mL of liquid Yeast Glucose Medium in 250 mL Erlenmeyer flask. Initial pH of the flask cultures was adjusted to 5.5 and then the flasks were incubated at 28±1°C for 15 days.

Extraction of lovastatin

For this purpose the methodology given by Lakshmanan and Radha (2012) was followed. To begin with 15 days old cultures of both strains were taken and sonicated for an hour in ultrasonicator bath (EN 30US) by which the fungal cells were lysed. The pH was adjusted to 3.0 using 0.001N HCl and an equal volume of ethyl acetate was added and kept in a rotary shaker for 2 hrs and then cooled for 12 hours in a refrigerator. The organic layers were separated using cold (4°C) centrifugation (REMI C24-BL) at 3000g for 20 minutes. The organic layers were concentrated under vacuum using rotavapour (BÜCHI Rotavapour R-205) at a temperature of 55°C. The concentrated samples were analyzed for the presence of lovastatin.

Analysis using UV-spectrophotometer method

The methodology given by Lakshmanan and Radha (2012) was followed. The presence of lovastatin was evaluated using UV-spectrophotometer. At regular intervals of time 1mL of samples were taken and centrifuged at 3000g for 20 mins, supernatant was taken and diluted with acetonitrile and the absorbance was measured. Maximum absorbance was obtained at 238 nm. Calibration chart was prepared using standard lovastatin by dissolving with 1 mL ethanol and made up to a volume of 5 mL using distilled water. Various concentrations of the standard were taken and the absorbance was scanned over the range of 190 nm to 300 nm. The maximum absorbance was obtained at 238 nm which is the actual absorbance peak for lovastatin.

Analysis using high performance liquid chromatography

Here also the methodology given by Lakshmanan and Radha (2012) was followed. For this purpose 1.5 mL of the organic phase was completely evaporated in each case and the dried residue was dissolved in 1.5 mL of acetonitrile. All the samples were then filtered through 0.45 µm millerx before injection. The mobile phase was a mixture of acetonitrile and water (60:40 v/v) which was acidified with orthophosphoric acid to a concentration of 0.1 %. The flow rate was maintained at 1.5 mL/min throughout the run and the detection was carried out at 238 nm.

RESULTS

Analysis using UV-spectrophotometer

Based on the absorbance of the spectrophotometer, the graph obtained for standards at different concentrations were stacked and represented in Fig. 1. Various concentrations of the standard were prepared and the absorbance was scanned over the range of 190 nm to 300 nm using UV spectrophotometer. The maximum absorbance was obtained at 238 nm which represent the actual absorbance peak for lovastatin. Absorbance was noted using the extract of pure culture of *P. cystidiosus* and *P. sapidus* for the presence of lovastatin. The extracts used for both species also gave maximum absorbance around 238 nm (Figs. 2 & 3). This is a clear indication of the presence of lovastatin in the prepared extracts of both the *Pleurotus* species. So as to confirm it, the samples were also evaluated for the presence of lovastatin through HPLC analysis.

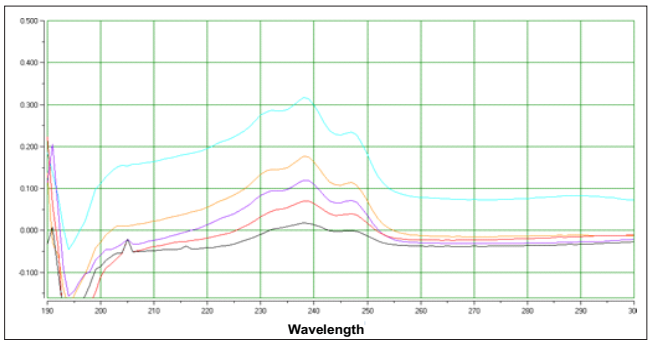

Fig. 1: Absorbance spectra of lovastatin standard at different concentrations

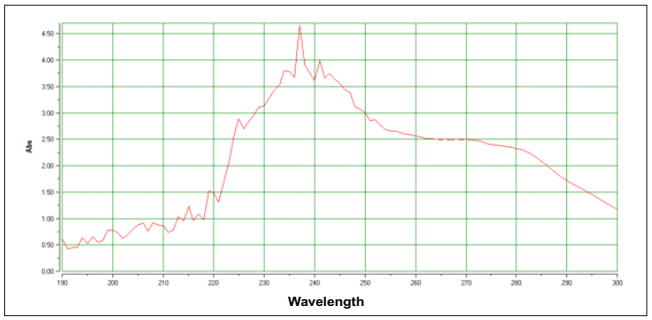

Fig. 2: Absorbance spectra of *Pleurotus cystidiosus* extract

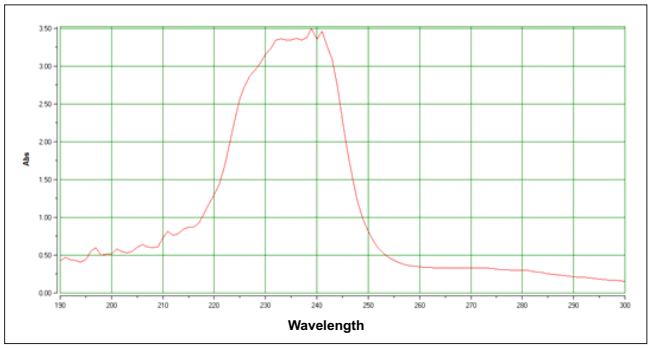

Fig. 3: Absorbance spectra of *Pleurotus sapidus* extract

Analysis using HPLC

Analysis of lovastatin in the pure culture of *P. cystidiosus* and *P. sapidus* was also carried out by the HPLC method. Extracted sample and standard showed similar peak area ranges and retention times which indicated the presence of lovastatin in the evaluated samples as shown in Figs 4-7.

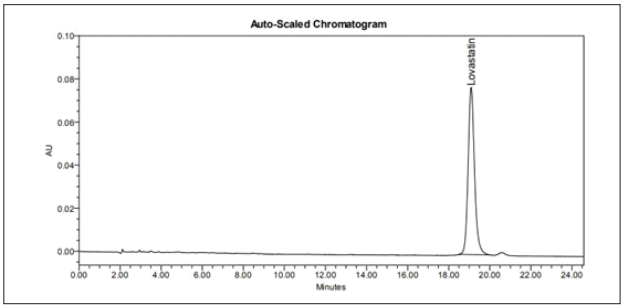

Fig. 4: HPLC chromatogram of standard of lovastatin

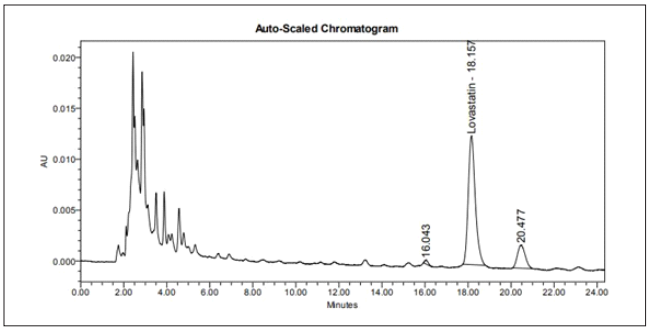

Fig. 5: HPLC chromatogram of lovastatin composition in *P. cystidiosus*

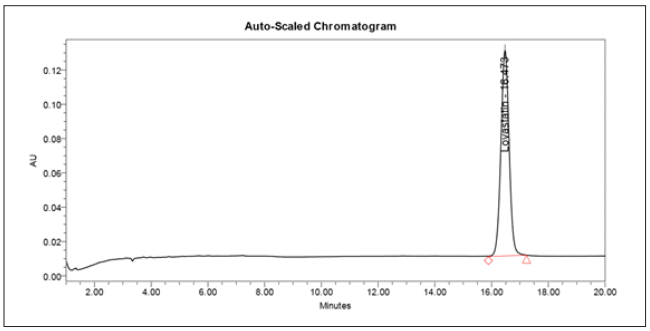

Fig. 6: HPLC chromatogram of standard of lovastatin

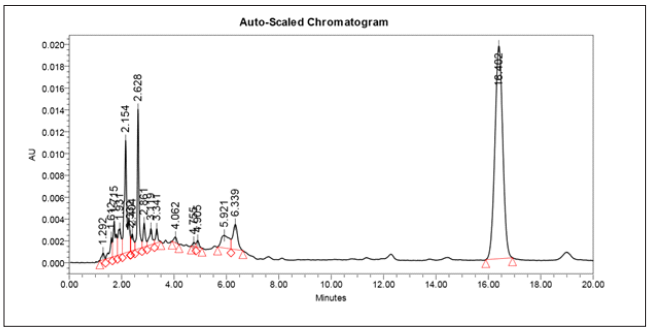

Fig. 7: HPLC chromatogram of lovastatin composition in *P. sapidus*

Chromatogram analysis indicated the peak of lovastatin standards with the RT (retention time) of 19.090 and 16.473 minutes. The extracts of the fungal samples were eluted at RT of 18.157 minute for *P. cystidiosus* and RT of 16.402 minutes for *P. sapidus* which were within the range of the retention times obtained for standards. Quantitatively the amount of lovastatin is calculated at 8.50 µg/mL in *P. cystidiosus* and 8.41 µg/mL in *P. sapidus*.

DISCUSSION

Lovastatin is reported to be an important constituent of number of different fungal taxa including *Aspergillus, Gymnoascus, Hypomyces, Monascus, Paecilomyces, Penicillium, Phoma, Phythium, Pleurotus, Scopolariopsis, Trichoderma*, etc. (Szakacs *et al.,* 1998; Manzoni *et al.,*1999; Manzoni and Rollini, 2002; Bizukojc and Stanislaw, 2009; Cabral *et al.,* 2010). In consonance with the present findings, the presence of lovastatin in *Pleurotus* has been reported by Gunde *et al.* (1973) and Alarcon *et al.* (2003). According to The Expert Panel (1998) this compound is reported to help in the lowering of cholesterol concentration in blood; particularly bad cholesterol (low density lipoprotein, LDL) while it is reported to increase the significant concentration of good cholesterol (high density lipoprotein, HDL). Thus it prevents the formation of plaque accumulation inside the arteries and further helps in prevention of cardiovascular diseases (Kavalipati *et al.,* 2015). In view of this it is important to look for newer sources of this nutraceutically important compound.

The literature regarding the production of lovastatin from higher basidiomycetes fungal strain is limited. The results of the present study revealed the presence of lovastatin in *Pleurotus cystidiosus* and *P. sapidus*. Both the mushrooms are well known for their edibility and nutraceutical credentials (Atri *et al.,* 2012, 2013, 2019; Kaur and Atri, 2019). The results of the present investigations are in conformity with the findings of Alarcon *et al.* (2003) and Jaivel and Marimuthu (2010) who reported the presence of lovastatin in *P. ostreatus*. Alarcon and Aguila (2006) also documented the presence of lovastatin in two strain PLUBB-127 (4.15 mg/L) and strain PL-136 (43 mg/L) of *P. ostreatus*. Chen *et al.* (2012) also reported the presence of lovastatin in the fruiting bodies of *P. ostreatus* (60.65 mg/100g dw), *Agaricus bisporus* (56.54 mg/100g dw) and *Boletus edulis* (32.73 mg/100g dw). Lin *et al.* (2013) evaluated lovastatin content in the mycelium of *P. ostreatus* at 2.22 mg/100g dw. Radha and Lakshmanan (2013) reported the presence of lovastatin in different mushrooms, namely *P. ostreatus, P. saca* and *P. sapidus.* Katarzyna *et al.* (2020) while working with *Agaricus bisporus, Cantharellus cibarius, Imleria badia* and *Lentinula edodes* evaluated largest amount of lovastatin in the fruiting bodies of *Cantharellus cibarius* (67.89 mg/100 g dw), and the smallest in of *Lentinula edodes* (0.95 mg/100 g dw). Ramakrishanan *et al.* (2017) also reported the presence of lovastatin in *P. ostreatus* (grey), *Hypsizygus ulmarius* (white) and *Agaricus djamor* (pink). As compared quantitatively the amount of lovastatin documented during the present study in *Pleurotus cystidiosus* (8.50 µg/mL) and *P. sapidus* (8.41 µg/mL) is not very high, but the mere presence of such an important medicinal constituent in them adds to their rich nutraceutical profile which is of definite advantage to the people suffering from high cholesterol in particular and mycophagist society in general.

CONCLUSION

The fruiting bodies of edible mushrooms, their mycelium and isolated extracts from them serve as functional foods because of their nutraceutical advantages to the suffering people in general. Amongst various mushrooms the production variable amount of lovastatin is well known. The documentation of lovastatin in *P. cystidiosus* and *P. sapidus* through UV-spectrophotometer and chromatographic analysis is an important finding. These two species of *Pleurotus* can also serve as potential material for genetic engineering and biotransformation so as to increase the yield of lovastatin in them.

ACKNOWLEDGEMENTS

Authors are thankful to Head, Department of Botany, Punjabi University Patiala (Punjab), India for providing necessary laboratory facilities and to University Grants Commission, New Delhi for providing financial support under BSR fellowship and DRS (SAP) program.

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