Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

AN EFFECTIVE QUANTITATIVE ESTIMATION OF LOVASTATIN FROM *PLEUROTUS OSTREATUS* USING UV AND HPLC

D.LAKSHMANAN AND K.V.RADHA*

Department of Chemical Engineering, A C College of Technology, Anna University, Chennai 600025. Email: radhavel@yahoo.com

Received: 14 May 2012, Revised and Accepted: 27 Jun 2012

ABSTRACT

Lovastatin is a best known drug, which effectively inhibits cholesterol biosynthesis. In this study, lovastatin was produced from *Pleurotus ostreatus* by solid state fermentation process. Lovastatin was quantified by microbial assay using *Candida albicans* in the culture extracts of *Pleurotus ostreatus*. Maximum yield of 113µg/ml was obtained with wheat bran as solid substrate, followed by rice bran, rice straw and sugarcane bagasse. In chickpea shells, no yield and growth was found. Presence of lovastatin was confirmed by high pressure liquid chromatography where lovastatin was eluted out at 4.3min.

Keywords: *Pleurotus ostreatus*, Lovastatin, UV-analysis, HPLC chromatogram.

INTRODUCTION

Lovastatin, also known as monocolin k, is an inhibitor of cholesterol biosynthesis, produced by *Pleurotus ostreatus* (*P.ostreatus*) as a secondary metabolite[1]. It inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), an important step in cholesterol biosynthesis[2]. Lovastatin is therapeutically used both in free acids and lactone forms[3]. β-hydroxic acid forms of lovastatin are insoluble in water, whereas it is soluble in lactone ring form. Lovastatin not only reduces blood cholesterol[4], but also has an anti-fungal property and anti-carcinogenic effects[5], which is proved by inhibiting the growth of *Rhodotorula rubra*[6], *Mucor racemosus*[7]. The growth of *Candida albicans (C.albicans)* also inhibits the β-hydroxic acid form of lovastatin.

Lovastatin is produced by both submerged and solid state fermentation process. Solid state fermentation (SSF) has more advantageous over submerged fermentation process as the process requires less power consumption[8]. A wide range of solid substrates can be used in SSF process which is mostly agricultural wastes. And the amount of water required in SSF is much lesser when compared with submerged fermentation process. Solid substrates have a greater influence in the production by solid state fermentation process[9].

In this study, different solid substrates were screened for the maximum yield of lovastatin from *P.ostreatus*. *C.albicans* was used in determining the concentration of lovastatin in the culture extracts.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade obtained from CDH (Central drug house, Chennai, India) including Potato dextrose agar (PDA), $MgSO_4.7H_2O$, $(NH_4)_2HPO_4$, NaCl. Various agricultural wastes such as wheat bran, rice bran, rice straw, sugarcane bagasse, oats meal, coconut shells, saw dust, corn, soya bean, chickpea shells was purchased from local market of Tamil Nadu, India. Lovastatin tablets was purchased from pharmacy in Chennai, Tamil Nadu, India and used as standard.

Microorganisms

The fungus, *P.ostreatus* was obtained from University of Madras, Tamil Nadu, Chennai. The stock cultures were maintained in Potato dextrose agar (PDA) at 4°C and were subcultured for every 15 days with PDA. The sub cultures were grown in Potato dextrose broth for 4 days and used as inoculum in solid state fermentation.

Screening of different substrates for lovastatin production

P.ostreatus was grown on different solid substrates (Wheat bran, rice bran, rice straw, Sugarcane bagasse, oats meal, coconut shells, Saw dust, corn, soya bean, chickpea shells). Ten grams of each solid substrates was taken separately in Petri plates and was moistened with distilled water containing MgSO4.7H2O (0.15g/l), (NH4)2HPO4 (0.25g/l), NaCl (1g/l) and steam sterilized at 121°C for 15min. The medium was cooled and inoculated with a four days old *P.ostreatus* culture. The culture flasks were then maintained at 28°C for 8 days.

Preparation of standard lovastatin

Lovastatin tablets of 10mg concentration was added with 10ml of acetonitrile and disintegrated by sonication for 10min which was followed by filtration using Whatman filter paper No. 1 and concentrated under nitrogen environment[10]. Different concentrations of lovastatin was prepared and analyzed by microbial method using *C.albicans*.

Extraction of lovastatin

P.ostreatus was incubated for eight days at 28°C. Later, plates were taken and dried at 60°C in hot air oven. From the dried culture, one gram of the culture was taken in a test tube, to which 10ml of ethylacetate was added. Lovastatin extraction was carried out by vortex agitation for 15min and was stored at cold condition for 1hour and centrifuged at 3000g for 15min. Supernatant was collected and bioassay was carried out using *C.albicans* to find the concentration of lovastatin present in the culture extract.

Bioassay with *Candida albicans*

C.albicans was grown on PDA for 12hrs at 28°C. *C.albicans* was subcultured on fresh PDA plates at a concentration of $7X10^3$ cells/ml and grown at 28°C. Fifty microliter of the extracts were taken and transferred to 6mm paper disk and placed on 90mm Petri plate containing *C.albicans*. The spacing between the control and the lovastatin were adjusted to be 15mm. Positive and negative controls were prepared by impregnating the paper with 50microliter of known concentration of lovastatin standard and ethylacetate respectively[11]. The plates were incubated for 12hrs and zone of inhibition was recorded.

Identification of lovastatin by HPLC method

The extract obtained from the culture was analyzed using HPLC. The extracts were dissolved in 100% acetonitrile solution, sonicated for two min and filtered through Whatman Filter paper No.1. A reverse phase high pressure liquid chromatography analysis were done using C18 column as a stationary phase. An isocratic condition was maintained in the mobile phase (acetonitrile and 0.1% of Phosporic acid (60:40 V/V)). The flow rate was maintained at 1.5ml/min throughout the run and the detection was carried out at 238nm. 20µl of the clear extracts was injected in Shimazudu high pressure liquid chromatography.

RESULTS AND DISCUSSION

Determination of lovastatin concentration by microbial method

Different concentrations of lovastatin were taken on a paper disk. The disk was placed in the agar plates containing *C.albicans* and a zone of inhibition was observed around the paper disk containing lovastatin. The diameter of the inhibition zone was correlated with different concentration of lovastatin taken in paper disk and as the concentration increases, the zone of inhibition also increased linearly (Fig.1). The slope solely depends on the concentration of agar present. As the concentration of agar increases, the diffusion rate decreases where it affects the formation of the inhibition zones. This study suggests that 2% of agar was found to be optimum, which gave a clear zone of inhibition.

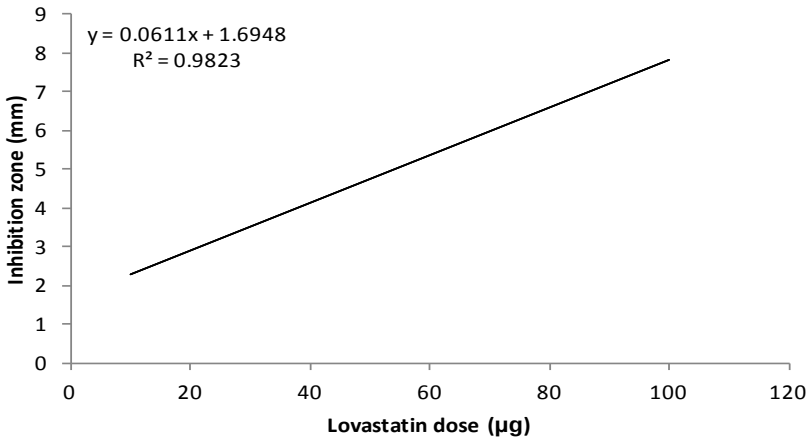

Fig. 1: Standard curve for the dose of lovastatin vs inhibition zone on the *C.albicans* plates

Yield of lovastatin using *Pleurotus ostreatus* by solid state fermentation on different substrates

P.ostreatus was grown on different solid substrates including wheat bran, rice bran, rice straw, sugarcane bagasse, oats meal, coconut shells, saw dust, corn, soya bean, and chickpea shells. Table1 shows the results obtained for various substrates. Wheat bran gave a maximum yield of 113µg/ml, whereas no yield was obtained from chickpea shells. The next higher yield was obtained from rice bran which gave a value of 63µg/ml .Wheat bran was easily utilized by the *P.ostreatus* and the growth was maximum when compared with other substrates. Followed by wheat bran and rice bran, rice straw and sugarcane bagasse gave a considerable yield, where coconut shell and saw dust gave low yield of production of lovastatin. Eventhough the growth was good in these substrates; there was a low yield which may be due to the presence of high lignocelluloses content in the substrates that may affect the formation of lovastatin as a secondary metabolite. In chick pea shell, both the growth and the yield were not found and hence it can be deduced that they are not suitable for the production of lovastatin from *P.ostreatus*.

Table 1: Yield of lovastatin on various solid substrates using *P.ostreatus*

S.No	Solid substrates	Inhibition zone (mm)	Yield of Lovastatin (µg/ml)
1	Wheat bran	8.4	113
2	Rice straw	4.1	41.5
3	Rice bran	5.4	63
4	Sugarcane bagasse	3.6	31.1
5	Corn	2.4	18
6	Oats meal	2.1	12
7	Saw dust	1.8	8
8	Coconut shells	0.8	4
9	Soya bean	1.3	6.3
10	Chickpea shells	0	0

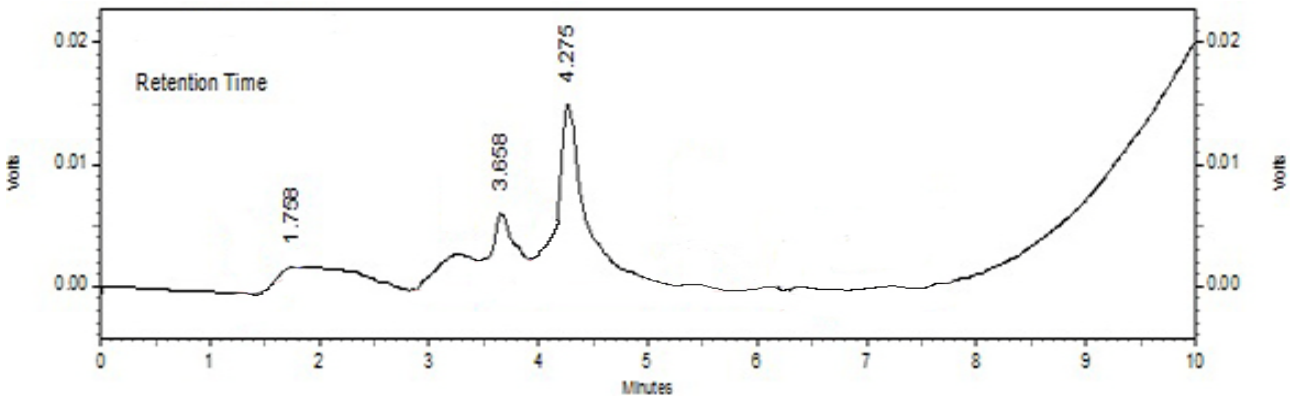

Fig. 2: Chromatogram of lovastatin obtained from *P.ostreatus* extracts

Analysis of *Pleurotus ostreatus* extract by HPLC

Analysis of lovastatin in the *P.ostreatus* extracts was carried out by HPLC method. Lovastatin was identified as β-hydroxic acid in the culture extracts of *P.ostreatus* (Fig.2). Identification of lovastatin in β-hydroxic acid form was much easier than lactone form, since the β-hydroxic acid was very stable than the nascent lactone ring form.

The extracts were eluted out at a retention time of 4.3min which were much similar to the retention time obtained for the standard. Both the standard and the sample were eluted at same conditions in C18 column of HPLC. The elution obtained at 1.76min confirms the lovastatin in its nascent form. The peak obtained at 3.561min is due to the degradation of the lovastatin in lactone form. Lovastatin present both in β-hydroxic acid and lactone form in the extracts were best studied using HPLC.

CONCLUSION

The present study concludes that the lovastatin was produced from *P.ostreatus* by HPLC. The yield was maximum when wheat bran was used as a solid substrate for the production of lovastatin. But there was no yield and growth when chickpea shell was used as a solid substrate. This concludes that lovastatin production can be carried out using wheat bran as solid substrate using *P.ostreatus*.

REFERENCE

1. Shami ARL, Hamed ER. Production of Lovastatin by *Pleurotus Ostreatus*. Egypt. J. Biotechnol 2007; 25: 102-109.
2. Punitha S and Senthil Kumar KL. Statin therapy and their formulation approaches: A review. Int J Pharm Pharm Sci 2011; 3: 41-44.
3. Chakravarti R, Sahai V. Compactin—a review. Appl Microbiol Biotechnol 2004; 64: 618–624.
4. Srinivas C, Abdul Bari Mohd, Sanayaima Huidrom, Padmananbha Rao, Prakash V Diwan. Development and validation of simvastatin in microemulsion formulation using RP-HPLC. Int J Pharm Pharm Sci 2012; 4: 398-403.
5. Van de Donk NW, Kamphuis MM, Lokhorst HM, Bloem AC. The cholesterol lowering drug lovastatin induces cell death in myeloma plasma cells. Leukemia 2002; 16: 1362–1371.
6. Baranova NA , Kreiner VG , Egorov NS. The growth of *Rhodotorula rubra* yeasts and their synthesis of ergosterol on media with lovastatin. Antibiot. Khimioter 1996; 41: 3-6.
7. Roze LV, Linz JE. Lovastatin triggers an apoptosis-like cell death process in fungus *Mucor racemosus*. Fungal Genet. Biol 1998; 25: 119–133.
8. Reeta Rani Singhania, Anil Kumar Patel, Carlos R. Soccol, Ashok Pandey. Recent advances in solid-state fermentation. Biochem. Eng. J. 2009; 44: 13-18.
9. Catherine Hazel M Aguilar, Joey Real Marie I Barbosa, Florence Lasalita Zapico, Remedios S Flamiano. Growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) feeding on different agro-industrial wastes. USM R & D 2010; 18: 73-76.
10. Casas JL, Sánchez JA, Sevilla JMF, Acién Fernández FG, Molina Grima E, Chisti Y. Production of lovastatin by *Aspergillus terreus*: effect of C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb. Technol. 2003; 33: 270-277.
11. Vilches Ferrò MA, Casas Lòpez JL, Sánchez Pérez JA, Fernández Sevill JM and Chisti Y. Rapid screening of *Aspergillus terreus* mutants for overproduction of lovastatin. World J. Microbiol. Biotechnol. 2005; 21: 123-125.