**Research Article**

# Evaluation of the effectiveness of soil fungi in the bioconversion of lovastatin to simvastatin and optimization of the cultivation medium

**Bhargavi Gangadhar[1], Savitha Janakiraman[2], and Nataraju Angaswamy[1*]**

*[1]Department of Studies and Research in Biochemistry, Karnataka State Open University, Mysuru, Karnataka*
*[2]Department of Microbiology and Biotechnology, Bangalore University, Bangalore, Karnataka*

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Corresponding Author: ****Nataraju Angaswamy***. Email: natabiochem@gmail.com

## ABSTRACT

**Introduction and Aim:** Simvastatin, a cholesterol-lowering drug, was manufactured industrially utilizing a multi-step semi-synthetic approach using lovastatin, naturally derived from *Aspergillus terreus*. Biocatalytic techniques are also used as a viable alternative. We provide an inexpensive mycological method for the bioconversion of lovastatin to simvastatin with easy downstream exertion.

**Methodology:** Overall, 35 fungi and 2 Actinomycetes sp. were tested for bioconversion by culturing them in potato dextrose broth with lovastatin (1mg/ml) for 7 days at 28°C with shaking (110 rpm). Analysis employing thin layers and high-pressure liquid chromatography revealed *Rhizopus sp*. and *Aspergillus sp.* as positive isolates. These isolates were cultured using different liquid media comprising lactose, Maida, corn steep, and nitrogen-limiting media, alongside a solid medium (wheat bran). Finally, the crude sample was purified using gradient elution column chromatography.

**Results:** Lactose medium and wheat bran produced the largest yields of simvastatin in both isolates, *Rhizopus sp.* (260 mg/L) and *Aspergillus sp.* (200 mg/L). Other media, on the contrary, produced a low yield (40 mg/L; 20 mg/L) for both isolates. The crude extract was purified using a 70:30 (benzene: acetonitrile, v/v) mobile phase, resulting in a maximum yield of 2.6mg/G dry weight with a measured purity of 96%.

**Conclusion:** Unlike existing technologies, the bioconversion technique based on soil fungus is cost-effective, allowing for easy downstream exertion with high yield. As a result, the current process gives researchers a new opportunity to obtain simvastatin effectively.

**Keywords:** Acetonitrile, *Aspergillus terreus*, Nitrogen limiting media, *Rhizopus sp.*, Wheat bran.

## 1. INTRODUCTION

Cholesterol, a lipid sterol is found exclusively in animals and also supplied in diet; hence it is often called an animal sterol. It plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein is recognized as ‘good cholesterol’ and low-density lipoprotein is considered as ‘bad cholesterol’[1].

High cholesterol concentration in the blood plasma is a leading risk factor for human cardiovascular diseases such as coronary heart disease and stroke generally termed atherosclerosis. Various medicines are available for lowering blood cholesterol levels, which may be prescribed individually or in combination with other drugs. Major among which are ‘statins. Statins lower LDL and triglycerides and have a mild effect in raising HDL, with a few short-term side effects. They work by interrupting the formation of cholesterol from the circulating blood [2].

The statin class of drugs is the first line of treatment for most people with high cholesterol. Therefore, these drugs are considered medically very important as they lower the serum cholesterol concentration by inhibiting “(3S) hydroxy 3, methyl-glutaryl coenzyme A (HMG CoA) reductase” a key enzyme in the cholesterol biosynthetic pathway which catalyzes the rate-limiting step in cholesterol biosynthesis, i.e.,

HMG CoA to mevalonate. Hence, statins are generally termed "HMG CoA reductase inhibitors" [3].

Examples of statins approved for use as medications include Simvastatin (Zocor), Atorvastatin (Lipitor), Fluvastatin (Lescol XL), Lovastatin (Mevacor, Altoprev), Pravastatin (Prav alcohol), Rosuvastatin (Crestor), and mevastatin (Mevacor). Statins have effects other than lipid reduction, called pleiotropic effects, such as anti-inflammatory, anti-microbial, anti-tumor, anti-hyperlipidemias, anti-thrombotic, anti-T cell adhesion, anti-oxidant, and other immune-modulatory activities. Among the statins lovastatin and simvastatin is the most prescribed drug in recent days with lesser side effects. Lovastatin belongs to a class of HMG CoA reductase inhibitors in humans and animals [4]. The Food and Drug Administration approved lovastatin isolated from the fungus *Aspergillus terreus* in August 1987. Lovastatin is typically produced within the fermentation conditions of the fungus.

Many studies have been evaluated proving that simvastatin shows good treatment results with very few side effects and a high antimicrobial activity compared to other statins [5]. The synthesis of simvastatin *in vitro* or *in vivo* is not straightforward as it involves a semi-synthetic approach starting from the natural product lovastatin.

The molecular difference between lovastatin and simvastatin resides in the side chain on the C 8 position. Here, lovastatin carries a 2-methyl butyrate moiety, while simvastatin has a 2, 2-dimethylbutyrate (DMB) moiety in this position. The main route of simvastatin synthesis is involved with de-esterification of the 2-methyl butyrate side chain of lovastatin, followed by several distinct chemical steps that involve lactonization, to yield a key intermediate monacolin J, with subsequent hydroxy group protection/deprotection. The protected monacolin J is then subjected to acylation by dimethyl butyrate chloride to yield the protected form of simvastatin, which is subsequently deprotected to yield pure simvastatin i.e., re-esterification with the appropriate 2, 2-dimethyl butyrate side chain at Carbon-8 positions of monacolin J. This process results in a low overall yield. Few other methods described involve the conversion of lovastatin to simvastatin using fewer chemical steps mainly involving acyl transferases. However, even this method employs expensive chemical reagents and also results in low overall yield. Recently, scientists have developed a one-step, whole-cell biocatalytic process for the synthesis of simvastatin from monacolin J [6, 7].

Another and the most convenient method is microbial bioconversion. Bioconversion is the process of chemical modification (or modifications) made by an organism (microorganism) on a chemical compound, yielding a new one with minor modifications with the help of some specific enzymes present in the organism. When bioconversion results in metabolites of lower toxicity, the process is known as detoxification. Most bio-transforming enzymes are high molecular weight proteins, composed of chains of amino acids linked together by peptide bonds. A diversity of bio-transforming enzymes exists and most of them catalyze the reaction of only a few substrates, meaning that they are highly "specific" in action [8, 9].

## 2. MATERIALS & METHODS

### Materials:

Lovastatin (>98% HPLC) was purchased from Sigma Chemical Co. USA (KM017963), dissolved in ethyl acetate (1mg/ml), and stored at 4°C before use. Simvastatin (>98%HPLC) was purchased from Sigma Chemical Co. USA (S6196 25MG), dissolved in ethyl acetate (1mg/ml), and stored at 4°C before use. All other chemicals, glass wares, and reagents used were of analytical grade.

### Microbial cultures:

Soil Fungi - Soil samples were collected aseptically in sterile containers from various locations across Karnataka, brought to the laboratory, and serial dilution was performed (Booth., 1971). The serially diluted (0.1ml) soil samples ($10^5$ and $10^6$) were plated onto PDA (potato dextrose agar) and incubated at 28°C for 5 to 7 days until fungal growth was observed.

Every isolate was sub-cultured and maintained on PDA slants for further work.

*Aspergillus terreus* (lovastatin-producing organism) was obtained from the microbiology laboratory, at Bangalore University; sub-cultured and maintained on PDA slants for further work. Two Actinomycetes cultures were obtained from the microbiology laboratory, at Bangalore University; sub-cultured and maintained on NA slants for further work.

**Screening and assay for bioconversion:**

**Submerged fermentation**:

Five types of growth media were used for the bioconversion process (compositions as mentioned in Table 1). The protocol followed is given below;

**Table 1: List of liquid medias used for bioconversion**

| |
|---|
| **Media No.1: Potato Dextrose Broth, Grams/1000ml:** Potatoes: 200 (Sliced washed unpeeled, Dextrose: 20, pH: 5.5 ± 0.2. |
| Preparation: Potato infusion can be made by boiling 200 grams of sliced potatoes in ~ 1000ml of distilled water for 30 minutes and then straining the broth through cheesecloth. Distilled water is added such that the total volume of the suspension is 1000ml. 20g dextrose is then added and the medium is sterilized by autoclaving. |
| **Media No. 2: Lactose Broth, Grams/1000ml:** 30g of Lactose in 1000ml Potato Dextrose broth (PDB). pH: 5.5 ± 0.2. |
| **Media No. 3: Corn Steep Broth: Grams/1000ml:** Corn steep liquor: 41.40, Soya peptone: 3.06, Commercial sucrose: 10.0, Ammonium sulfate: 2.0, Yeast extract: 2.0, $KH_2PO_4$: 0.85, $CaCl_2$, $2H_2O$: 0.1, $MgSO_4$, $7H_2O$: 0.1, NaCl: 0.1, pH: 5.5 ± 0.2. |
| **Media No.4: Nitrogen Limiting Broth Grams/1000ml:** Glucose: 10.0, $KH_2PO_4$: 2.5, $CaCl_2$: 0.1, Yeast extract: 5.0, $MgSO_4$, $7H_2O$: 0.5, $FeSO_4$, $7H_2O$: 0.02, $KNO_3$: 1.0, $MnSO_4$: 0.01, $CuSO_4$, 5 $H_2O$: 0.002, $ZnSO_4$, $7H_2O$: 0.01, pH: 5.5 ± 0.2. |
| **Media No.5: Maida Broth, Grams/1000ml:** Maida: 41.40, Soya peptone: 3.06, Commercial sucrose:10.0, Ammonium sulfate: 2.0, Yeast extract: 2.0, $KH_2PO_4$: 0.85, $CaCl_2$, $2H_2O$: 0.1, $MgSO_4$, $7H_2O$: 0.1, NaCl: 0.1, pH: 5.5 ± 0.2 |

Seven days old fungal spores were suspended in sterile distilled water and aseptically inoculated (50ul of inoculums volume) in a 250ml flask containing 100ml of growth medium along with 60µl of lovastatin as substrate and 60µl of olive oil (pH 5.5±0.2). The flasks were incubated on a Rotary shaker at 125rpm, 26 ± 2ºC for 7 days. A control i.e., culture medium with substrate and oil but, without microorganisms was incubated concurrently under the same conditions for 7 days, after which the cultures were filtered and extracted with ethyl acetate (at 1:1 volume ratio), the extract was then analyzed by, TLC and HPLC in comparison with standard lovastatin and Simvastatin [10] [11].

**Solid-state fermentation:**

Wheat bran was used as a solid substrate for the bioconversion of lovastatin to simvastatin. Two grams of the substrate was taken in a 100ml flask and the relative humidity was maintained at 70%. One ml of spore suspension and 30µl of lovastatin substrate with 30µl of olive oil was added to the substrate post-autoclaving and incubated at 28°C for 7 days (in triplicates), [12].

**Extraction of Simvastatin:**

**SmF:** Fungal organisms showing the highest titer of simvastatin were inoculated into 500ml Erlenmeyer flasks containing 150ml of Liquid medium along with 0.1ml of lovastatin substrate and 0.1ml of olive oil (in triplicates) and incubated at 28°C in a rotary shaker at 110 rpm. After 7 days of incubation, the mycelia were filtered using Whatman N0.1 paper. The filtrate was extracted with ethyl acetate (at a 1:1 volume ratio) using a separating funnel and was concentrated by vacuum evaporation overnight. The crude extract contained one main metabolite i.e., simvastatin analyzed through TLC and was then subjected to a purification process [13,14].

**SSF:** Fungal organisms showing the highest titer of simvastatin were inoculated into 500 ml Erlenmeyer flasks containing 20gms of wheat bran along with 0.1ml of lovastatin substrate and 0.1ml of olive oil (in triplicates) and incubated at 28°C for 7days (with 70% relative humidity). After incubation, 50ml of ethyl acetate was added to each flask and kept in a shaker (at 28°C, 110rpm) for 1 hour followed by filtration through Whatman No.1 filter paper. The filtrate was concentrated through vacuum evaporation overnight. The crude extract contained one main metabolite i.e., simvastatin analyzed through TLC and was then subjected to a purification process [13,14].

**Thin layer chromatography:**The crude extract diluted with 100µl of ethyl acetate was spotted onto heat-activated 20×20 cm silica gel TLC plates. The mobile phase used was dichloromethane and acetate (7:3, v/v). All the plates were observed under a hand-held UV lamp (254ηm) after developing in the same mobile phase three times and then exposed to iodine vapor. For each TLC run, the retention factors of both lovastatin and simvastatin authentic

standards were compared with the samples for confirmation [15]

**High-pressure liquid chromatography:** Extracted simvastatin was membrane filtered using ≤1.0ml ethyl acetate into a fresh vial and HPLC analysis was carried out. For HPLC, an ODS (250mm×4.6mm I.D Micrometer) column with a diode array detector was used. The mobile phase used was acetonitrile and water (containing 0.1% ortho-phosphoric acid) with a volume ratio of 70:30 respectively. The eluent flow rate was maintained at 1.5ml per minute and detection was carried out at 235ηm with an injection volume of 20µl. Authentical grade simvastatin was used to prepare the standard for HPLC analysis [15].

**Purification of simvastatin:** Simvastatin purification was carried out by an overloaded elution chromatography column using silica gel as the stationary phase with a mesh size of 60/120 (length of the column was 35cm) followed by the gradient method. A mixture of benzene and acetonitrile was used as mobile phase with a dwell time of 40 50 drops per minute. Sample with all gradient ratios ranging from 100, 90:10, 80:20, and 70:30 (v/v of benzene and acetonitrile respectively) was further analyzed by TLC, and the suitable gradient was confirmed with HPLC in comparison to standard simvastatin for purity [15].

## 3. RESULTS

A total of 35 fungal organisms and two Actinomycetes sp. were screened for the bioconversion of lovastatin to simvastatin by growing them in Potato Dextrose broth (PDB) supplemented with lovastatin (1 mg/ml) for 7 days under shaken condition (110 rpm) at 28°C. Morphological and microscopical observation (Figure. 1) revealed that the test fungal isolates belonged to the genus *Rhizopus*, *Aspergillus*, *Mucor*, *Penicillium*, *Alternaria*, and *Cladosporium*.

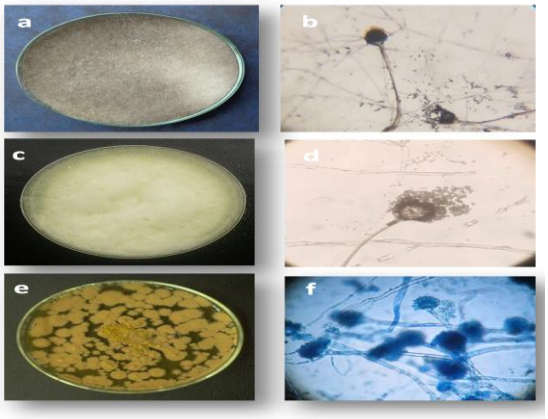


**Figure. 1 – Colony morphology and microscopic view of fungal isolates: 1a and b - *Rhizopus sp.*, 1c and d – *Aspergillus sp.*, 1 e and f – *Aspergillus terreus***

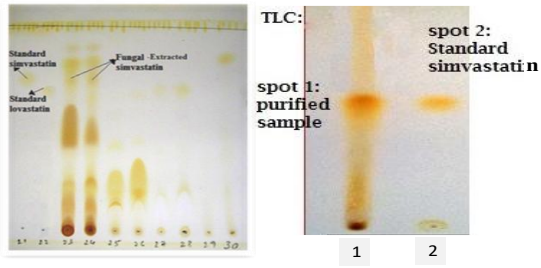


**Figure. 2 - Thin layer chromatography of Crude (left) and purified (right) Simvastatin.**

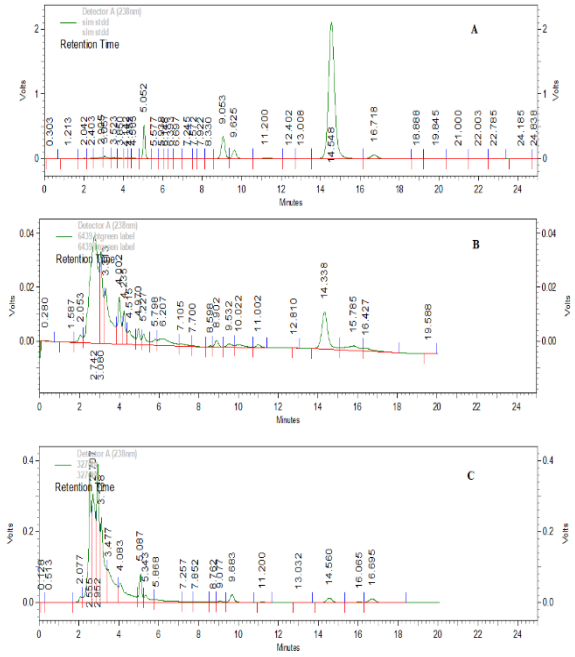


**Figure. 3 - HPLC chromatogram of - (A) standard simvastatin, (B) simvastatin obtained from *Rhizopus sp.*, and (C) simvastatin obtained from *Aspergillus sp.***

Out of 35 fungal isolates, only two, belonging to genus *Rhizopus sp.* and *Aspergillus sp.* showed positive for the transformation of lovastatin to

simvastatin as confirmed by TLC (thin layer chromatography) (Figure. 2) and HPLC (High pressure liquid chromatography) (Figure. 3) analysis with a single peak corresponding to standard simvastatin, these two isolates were used for further work.

The two positive simvastatin-producing isolates *Rhizopus sp.* and *Aspergillus sp.* were tested by growing on different media, including both SmF (Lactose broth, NLM, Maida media, and corn steep media) and SSF (Wheat bran) for the study of optimization of culture media and to increase the transformation efficiency.

Among the five liquid media used, lactose medium gave the highest yield in both the isolates i.e., *Rhizopus sp.* yielded 260 mg/L and *Aspergillus sp.* yielded 280 mg/L of simvastatin, followed by corn steep medium (with a yield of 160 mg/L and 120 mg/L respectively) and potato dextrose medium (with a yield of 40 mg/L and 60 mg/L respectively). In nitrogen-limiting media and maida media, both organisms did not show any increase in simvastatin yield. The addition of lactose significantly showed an increase in the yield of simvastatin (Figure. 4).

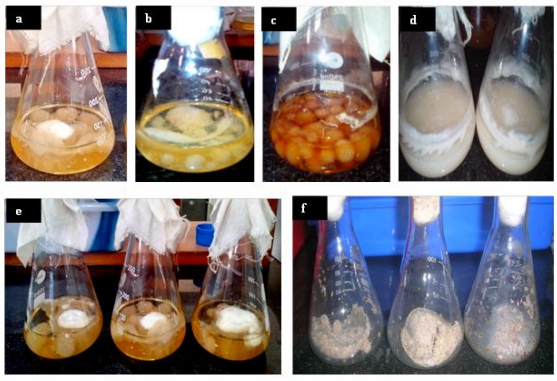


**Figure. 4 - Growth of *Rhizopus sp.* on different media a. Potato dextrose broth, b. Lactose broth, c. Corn steep broth, d. Maida media, e. Nitrogen limiting broth, f. SSF (Wheat bran as substrate).**

The crude simvastatin obtained by bioconversion was subjected to purification by gradient elution column chromatography. Among the four different ratios of mobile phases (benzene: acetonitrile, v/v i.e., 100:0, 90:10, 80:20 and 70:30), 70:30 (v/v) gave the highest yield of 2.6 mg/G DW of the substrate. The purity of simvastatin obtained was estimated as 96%.

## 4. DISCUSSION

Simvastatin as aforementioned is a cholesterol-lowering class of drug. During the US patent protection period, it was Merck & Co.'s largest-selling drug with a record of US $4.3 billion in sales in 2005. Since then, simvastatin has become available as a generic medicine and has become one of the top generic drugs in sales. Current industrial production of simvastatin is based on multiple steps; semi-synthetic modifications on purified lovastatin, a natural product from the fungus *Aspergillus terreus*. As in need of an efficient alternative, many researchers have attempted simvastatin production through various biocatalytic and bioconversion methodologies. Therefore, an attempt was made to bio-transform lovastatin to simvastatin with simple microbial techniques.

Out of 35 soil fungal isolates, only two, belonging to the genus Rhizopus and Aspergillus (Figure 1) showed positive for the transformation of lovastatin to simvastatin as confirmed by TLC (Figure. 2) and HPLC analysis (Figure. 3) with a single peak corresponding to standard simvastatin. These two isolates were used for further work. [10,16].

However, further confirmation through other analytical approaches is needed such as molecular mass determination by Liquid Chromatography/Mass Spectroscopy (LC/MS). It is known that carbon and nitrogen sources play a prominent role in fermentation productivity since these nutrients are linked with the formation of biomass and metabolites. For many years, bioconversion experiments have been extensively studied both by SSF solid state fermentation and SmF submerged fermentation processes where SmF with various percentages of carbon and nitrogen supplements has proved to provide a bioconversion favorable method, while SSF (Figure. 4) is a potential alternative of SmF in increased productivity and economical advantage [13]. In nitrogen-limiting media and Maida media, both organisms showed no increase in simvastatin yield. According to Nasmetova (2015), the addition of lactose significantly showed an increase in the yield of simvastatin by *Aspergillus terreus* strain 20, on the contrary same lactose media hypothesis did

not hold good results on the positive cultures we held. Our efforts in media formulation in the present work are directed to the improvement of the yield of simvastatin up to 96% with wheat bran as substrate.

## 5. CONCLUSION

Statins as per researchers have low-density lipoprotein lowering and cholesterol inhibition actions. The main aim of statin may not always be unidirectional but has several beneficial effects. Statins especially simvastatin has multiple pharmacological properties. In contrast to existing technologies, our bioconversion technique based on soil fungus is cost-effective since it uses solid waste as substrates, allowing for easy downstream work with high yield. As a result, bioconversion *via* a fungal process may give researchers, a new opportunity to get statins in a much more effective manner. In the future, diverse medicinal product development can use this safe and efficient procedure.

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## CONFLICT OF INTEREST

The authors state no conflict of interest.

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No funding was received for this research work.

## ETHICAL INFORMATION

No ethical information is involved in the research work.

## REFERENCES

1. Das P, Ingole N. Lipoproteins and their effects on the cardiovascular system. *Cureus*. 2023 Nov;15(11): e48865.
2. Peter L. The changing landscape of atherosclerosis. *Nature.* 2021 April; 592(7855): 524-533.
3. Ashrafizadeh H, Oroojan AA, Ahmadi I, An'aam M. The Role of Statins (HMG-CoA Reductase Inhibitors) on Insulin Secretion from the Islets of Langerhans: A Systematic Review. Jentashapir *Journal of Cellular and Molecular Biology*. 2022 Jun 30; 13(2): e129634.
4. Yu D, Liao JK. Emerging views of statin pleiotropy and cholesterol lowering. *Cardiovascular Research*. 2022 Feb 1; 118(2): 413-23.
5. Dhakal S, Macreadie IG. Simvastatin, Its Antimicrobial Activity and Its Prevention of Alzheimer's Disease. *Microorganisms.* 2024 Jun 1; 12(6): e1133.
6. Kant Belwal C, Patel J. Synthetic methods for simvastatin–An overview. *Journal of Pharmaceutical and Applied Chemistry*. 2019 Jan 1; 5(1): 1-21.
7. Tang XL, Yu JW, Geng YH, Wang JR, Zheng RC, Zheng YG. From Discovery to Mass Production: A Perspective on Bio-Manufacturing Exemplified by the Development of Statins. *Engineering.* 2023 May 1; 24: 138-50.
8. Balraj J, Murugesan T, Dhanapal AR, Kalieswaran et al. 2022 - Bioconversion of lovastatin to simvastatin by Streptomyces carpaticus towards inhibiting HMG–CoA activity. *Biotechnology and Applied Biochemistry*. 70 (3), 1162-1175.
9. García-Marquina G, Langer J, Sánchez-Costa M, Jiménez-Osés G, López-Gallego F. Immobilization and stabilization of an engineered acyltransferase for the continuous biosynthesis of simvastatin in packed-bed reactors. *ACS Sustainable Chemistry & Engineering.* 2022 Jul 19; 10(30): 9899-9910.
10. Nasmetova SM, Ruzieva DM, Rasulova GA, Sattarova RS et al. Effect of the principal nutrients on simvastatin production by wild strain Aspergillus terreus 20 in submerged fermentation. *International Journal of Current Microbiology and Applied Sciences.* 2015; 4(9): 894-898.
11. Salem WA, Elkady EF, Fouad MA, Mohammad MA. DoE screening and optimization of liquid chromatographic

determination of nicotinic acid and six statins: Application to pharmaceutical preparations and counterfeit detection. *Journal of Chromatographic Science.* 2023 Jan; 61(1): 74-86.

12. Fan D, Tang H, Zhao F, Yang X, Han S. Strategies for the statins production in microbial cell factory: present and future. *Authorea Preprints.* 2023 May 22. Available from: https://api.semanticscholar.org/CorpusID:259290528
13. Praveen VK, Savitha J. Solid State Fermentation: An Effective Method for Lovastatin Production by Fungi–A Mini Review. *The Open Tropical Medicine Journal.* 2012; 5: 1-5.
14. Ramadan AM, Shehata RM, El-Sheikh HH, Ameen F, Stephenson SL, Zidan SA, et al. Exploitation of sugarcane bagasse and environmentally sustainable production, purification, characterization, and application of lovastatin by Aspergillus terreus AUMC 15760 under solid-state conditions. *Molecules.* 2023 May 12; 28(10): 4048. Available from: https://doi.org/10.3390/molecules28104048
15. Ayothiraman S, Murugesan N, Sethi G. Critical analysis of analytical techniques developed for statins in biological fluids, environmental and fermentation samples. *Critical Reviews in Biotechnology*. 2024 Oct; 21: 1-31.
16. Lins UM, Mendonça RD, Dantas SS, Souza AF, Montero-Rodríguez D, Andrade RF, et al. Bioconversion of Alternative Substrates for the Biosynthesis of HMG-CoA Reductase Inhibitors by Aspergillus spp. Strains with Antimicrobial Potential. *Fermentation (Basel).* 2024 Jul 1;10(7). Available from: https://doi.org/10.3390/fermentation10070367