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Extraction and Purification of Lovastatin from the Edible Mushroom *Laetiporus sulphureus* and its Antioxidant Activity

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LOVASTATIN is a cholesterol-lowering drug produced by the secondary metabolism of different fungi. In the current study, lovastatin was extracted from the basidiomycetes fungus *Laetiporus sulphureus* by submerged fermentation using an organic solvent ethyl acetate . Initial detection of lovastatin production in the crude extract was carried out using agar well diffusion method and was found to have inhibitory activity against yeast *Candida albicans*. Thin-layer chromatography (TLC) was used to detected lovastatin, and a distinct spot with an RF value of 0.55 appeared similar to standard Lovastatin. A single peak was also featured with high-performance liquid chromatography (HPLC) with a retention time of 14.61 minutes to see the purity of Lovastatin. The result of the detection of active groups in lovastatin purified by the infrared spectrum indicated the presence of amide and carboxyl groups. The purified lovastatin showed antioxidant efficacy with the DPPH test, with the inhibitory effect reaching 75% at a concentration of 100μg/mL compared with the inhibitory effect of standard antioxidant, ascorbic acid, which reached 85%.

Keywords: DPPH assay, Edible mushroom, HPLC analysis, *Laetiporus sulphureus,* Lovastatin, TLC.

Introduction

Basidiomycetes produce many compounds that are instrumental in the progress of humanity towards improving health and eliminating some diseases and their effects and, Lovastatin is among the most important fungal products. This composite is produced by several fungal strains as secondary metabolites that are formed during the polyketide pathway. Lovastatin has high efficacy in lowering cholesterol levels in the blood, thus protecting people from heart disease and the risks associated with high blood cholesterol (Pandey et al., 2019). Lovastatin $C_{24}H_{36}O_5$ is also known as Monakolin K. Mellinolin and is the first statin to have been approved by Food and Drug Administration (FDA) in 1987 as a treatment for high cholesterol (Shivakumar & Ravurim, 2018).

An important step in the process of cholesterol formation in the body is mevalonate composition from 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA), by 3-hydroxy-3-methyl-glutaryl CoA reductase. Lovastatin binds to this enzyme and stops its action then cholesterol formation (Seenivasan et al., 2015).

It also works to reduce harmful cholesterol levels of low-density lipoprotein (LDL), and slightly raises the level of useful cholesterol levels of high-density lipoprotein (HDL) (Raj et al., 2019).

Advanced research revealed that lovastatin has an effective role in treating various diseases such as coronary heart diseases, renal diseases , Alzheimer's, bone fractures, etc., and many kinds of tumors within its capacity to suppress tumor growth *in vivo* (Praveen et al., 2014). The aim of this study is to extract lovastatin from a local fungal isolate from the Iraq environment and to test its antioxidant efficacy.

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Materials and Methods

Experiments related to this detailed study were conducted in the research unit of the Department of Biology/College of Education for Pure Sciences, and some others were accomplished at the Ministry of Science and Technology, Baghdad.

Sample collection

The collection of fungal samples was carried out in various sites of the farms extended on the banks of the Tigris River such as AL Rashidiyah, Al Kibbeh,AL Fadhiliya, Al-Nimrud Al Mosul Falls, Mosul Dam, Baashiqa, Hammam Al-Alil, and Mosul forests. They were entirely scanned over different periods of time. After collection, the samples were washed out with water and were left to dry out. Small pieces of fruiting bodies were taken and placed in ethanol 70% for several minutes. The sterilization was achieved with a 2% of sodium chlorate solution for 2min, then washed with distilled water and left to dry using sterile filter paper, then the samples were loaded onto PDA medium and incubated at 28 C° for 14 days (Kala et al., 2020). The samples were then tested for their ability to produce Lovastatin .

Production of lovastatin

The ability of fungal hyphae of *Laetiporus sulphureus* to produce lovastatin was tested using submerged fermentation in Soybean meal medium (Composition g\L: Sucrose- 50 , Soybean meal -20 , K_2HPO_4- 1 , $NaNO_3$- 1, $MgSO_4.7H_2O$- 0.5; pH6.5). The medium was distributed to 50 ml in each 250mL flask, and sterilized with an autoclave. Each flask was inoculated with three pieces of the colony of fungal hyphae that were taken with a cork borer 6mm . The flasks were incubated in a rotary shaker for 7 days at 170rpm (Shivakumar & Ravurim, 2018) .

Extraction of lovastatin

After the incubation period, samples were filtered. The filtrate pH was adjusted to break the bonds by 1N HCL to convert the lovastatin to β-hydroxy acid, after which a double amount of ethyl acetate is added. The samples were placed into the rotary shaker incubator for 24h, then centrifuged at 6,000rpm for 15 min. The samples were separated by a separating funnel (Fig. 1), where the organic top layer containing lovastatin was taken (Dhar et al., 2015).

Fig. 1. Separation of the organic layer using a separating funnel

One mL of trifluoroacetic acid (1%) was added to 1 ml of the supernatant and incubated for 10min, then 0.5mL of the incubated sample was taken and diluted 10 times with methanol. The absorbance was read at the wavelength 238 by UV-Visible Spectrophotometer (Raman et al., 2012).

Screening of lovastatin

The presence of lovastatin in fungus *Laetiporus sulphureus* extract was tested against standard test organisms *Candida albicans*. This was done by agar well diffusion method using cork borer 6 mm, and loading the wells with lovastatin extract, standard lovastatin as positive control and ethyl acetate as negative control, after inoculating the potato dextrose agar medium with *Candida albicans*, The medium was then incubated for 24 to 48h at 37°C to measure zone of inhibition (Atli & Yamac, 2012; Nidhiya et al., 2013).

Thinla yer chromatography(TLC) analysis

The presence of lovastatin was checked out using TLC plate silica gel (20 X 20cm), the silica gel sheet was placed into the oven at 80°C for a few minutes to activate it. Drops from the extract were applied into the standard silica gel. The mobile phase was prepared from dichloromethane and ethyl acetate (70:30 v/v), and it was repeated three times in the same mobile phase. The sample was observed under UV light iodine vapor (Praveen et al., 2014).

High performance liquid chromatography (HPLC) analysis

Lovastatin was purified using HPLC analysis by the C-18 column (250×4.6mm). The mobile phase consisting of 0.02 M phosphate buffer (pH 7.7) with acetonitrile in a ratio of 65:35 (v/v), 20mL of the sample was injected at a flow rate of 1.0mL\ min. The sample was detected at wavelength 238 nm, where the identity of the compound was confirmed with commercial lovastatin from the company Sigma- Aldrich (Janani et al., 2017). The remaining lovastatin was collected by fraction collectors.

Fourier-transform infrared spectrophotometer (FTIR)

The lovastatin sample was detected by means of instantaneous infrared transmission spectroscopy and compared with the standard lovastatin. The spectrum was scanned in a range from 4000cm^{-1} to 400cm $^{-1}$ using a detector DTGS KBr (Neduri et al., 2012).

Antioxidant efficacy

The radical scavenging colorimetric method was used with DPPH assay to determine the antioxidant activity of lovastatin. Methanol was used to dissolve the DPPH compound. A series concentration of lovastatin was prepared (12.5, 25, 50 and 100μg/mL). The standard antioxidant was ascorbic acid. Antioxidant effectiveness was estimated by mixing 1ml of DPPH with 1mL of each concentration of lovastatin. The reaction mixture was shaken vigorously and left in dark conditions at 28°C±2 for 30min. Absorbance DPPH was measured at the wavelength 517nm. Calculation of the percentage of inhibition by DPPH was conducted using the method described by Rengarajan et al. (2013) as the following formula:

$$\text{DPPH inhibition\%} = \frac{(\text{ABS control} - \text{ABS sample})}{(\text{ABS control})} \times 100,$$

where, ABS refers to absorbance.

Results and Discussion

The fungal extract of *Laetiporus sulphureus* showed a high inhibitory activity compared with standard lovastatin (Fig. 2). The diameter of the degradation zone was 30mm, while no inhibition was showed with the negative control (ethyl acetate) (Atli & Yamac, 2012). The growth inhibition mechanism of yeast is due to that lovastatin is in the form of β-hydroxy acid, which is known to be an effective antifungal compound. *Candida albicans* has a lipid bilayer in its cell membrane . Its cell wall is composed of sterols, which are the target of antifungal agents. The mechanism of inhibition of yeast could be due to interaction between lovastatin and the electronegative charges of the cell membrane which lead to a change in membrane permeability and consequently leakage of proteins and other intracellular electrolytes (Vilches feron et al., 2005; Khalil & Yousef, 2020). The much higher inhibition caused by the fungal extract may be attributed to presence of lovastatin and other biologically active secondary metabolites.

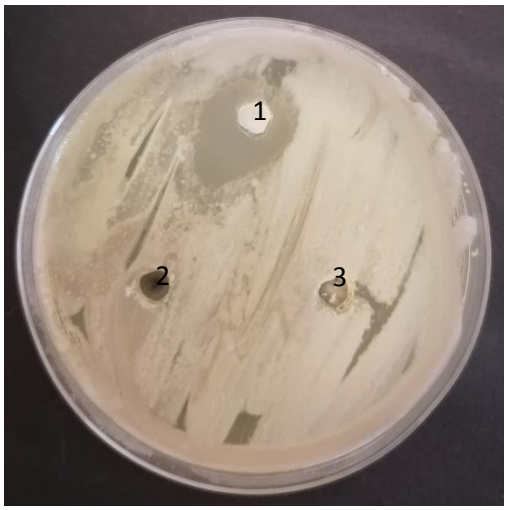

Fig. 2. Growth inhibition of *Candida albicans* by *Laetiporus sulphureus* extract [1- *Laetiporus sulphureus* extract, 2- Standard lovastatin, 3- Negative control]

The amount of lovastatin produced by *Laetioporus sulphureus* was estimated (280µg/mL). This amount is excellent when reviewing research that includes lovastatin production from various fungi as *Shizophyllum commune* and Pleurotus ostreatus which produced 38µg/ml and 30µg/mL , respectively (Pushpa et al., 2016).

TLC analysis

In order to establish whether the area of inhibition that appeared was due to the effect of lovastatin, a TLC analysis was performed.

The isolate extract of *Laetiporus sulfureus* has appeared in TLC when using short wavelength UV and iodine vapor. The fluorescence showed a distinct spot with an Rf value of 0.55, which is the same as the Rf value for standard lovastatin that was calibrated (Fig. 3). This indicates the presence of lovastatin in *Laetiporus sulphureus* extract (Jaivel & Narimuthu, 2010). The TLC analysis confirmed the growth inhibition of *Candida albicans* on the biological assay due to lovastatin activity. This result is consistent with (Mangunwardoyo et al., 2012), when studying bioprospect of lovastatin in *Aspergillus* spp.

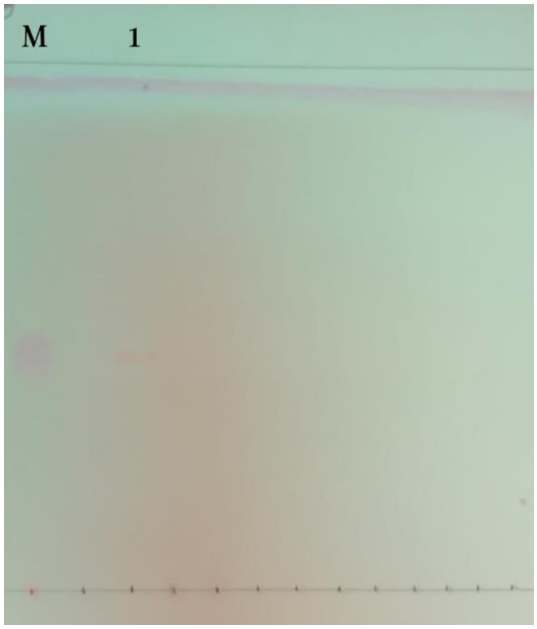

Fig. 3. TLC analysis [Lane M: Standard lovastatin, Lane 1: *Laetiporus sulphureus* extract

HPLC analysis

The HPLC analysis has confirmed the presence of lovastatin in studied samples, where a characteristic lovastatin peak appeared at a retention time of 14.61, while the standard retention time for lovastatin sample was 14.64 (Fig. 4). The appearance of a single peak in the HPLC analysis indicated the high purity of the extract (Goswami et al., 2013).

FTIR analysis

The analysis of infrared absorbance spectrum showed an absorption peak belonging to the N-H group at the frequency 3539.38, whereas the absorption peaks at frequencies 3014, 2962, 2927 and 2870, belong to C-H group, while the absorption peaks at frequencies 1718, 1458 and 1074 belong to N-O group, C=O and C-H group, respectively. The absorption peaks at frequencies below 1000 indicate heavy metals (Fig. 5) (Bhuvaneshwari & Anand, 2018).

DPPH radical scavenging activity

Due to the fact that ascorbic acid is known for its antioxidant efficacy, it was chosen as a positive control (Bhargavi et al., 2016). The results of the antioxidant activity of Lovastatin extracted from *Laetiporus sulphureus* showed gradual increases with increasing the concentration of lovastatin (Fig. 6). The maximum inhibitory activity was 75% at 100μg/mL, while the lowest inhibitory activity was 30% at the lowest concentration 12.5μg/mL. It is clear that lovastatin has a high antioxidant efficacy (Mohan et al., 2011; Lung & Huang, 2012). These results are consistent with Ajith et al. (2006) who indicated that lovastatin possess antioxidant efficacy, in their tagged research, Proapoptotic and antitumor activities of the HMG-CoA reductase inhibitor, lovastatin, against Dalton's lymphoma ascites tumor in mice. It is possible that the antioxidant activity of lovastatin is due to the presence of the functional groups in the structure of lovastatin, as lovastatin has a side chain methyl butyric acid and methyl group (Puttananjaiah et al., 2011). These results are consistent with Talkad et al. (2015) which lovastatin has antioxidant activity that interferes with the production and formation of free radicals and plays a major role in disrupting them and thus reduced the risk of cancer and cardiovascular disease, when studying lovastatin isolated from *Pleurotus ostreatus.*

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Conflict of interests: The authors declare no conflict of interest.

Authors contribution: Dr. Shimal Y Abdel-Hadi constituted by 50% (She suggested the idea, helped in the final revision); Othman A Mahmoud contributed by 50% (He collected the samples, wrote the final draft, helped in the final revision)

Ethical approval: Not applicable

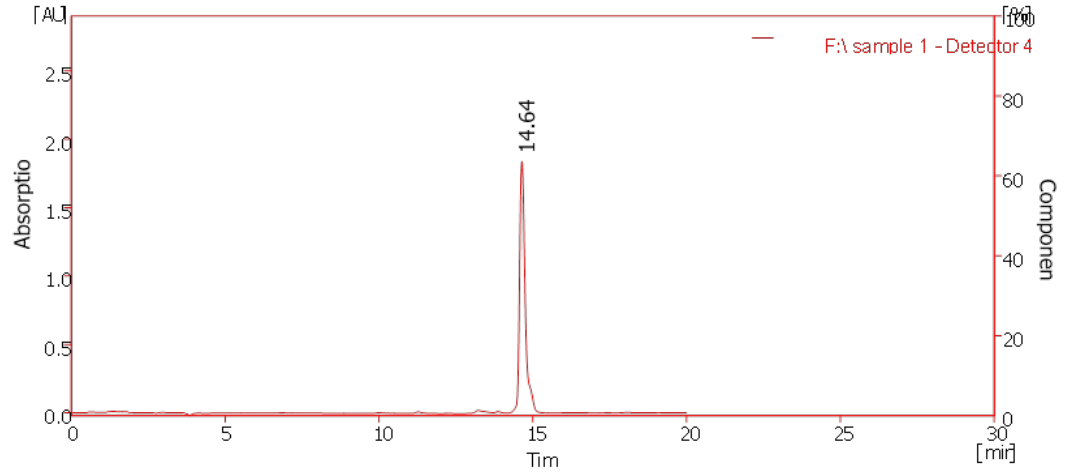

Fig. 4a. HPLC analysis of standard lovastatin

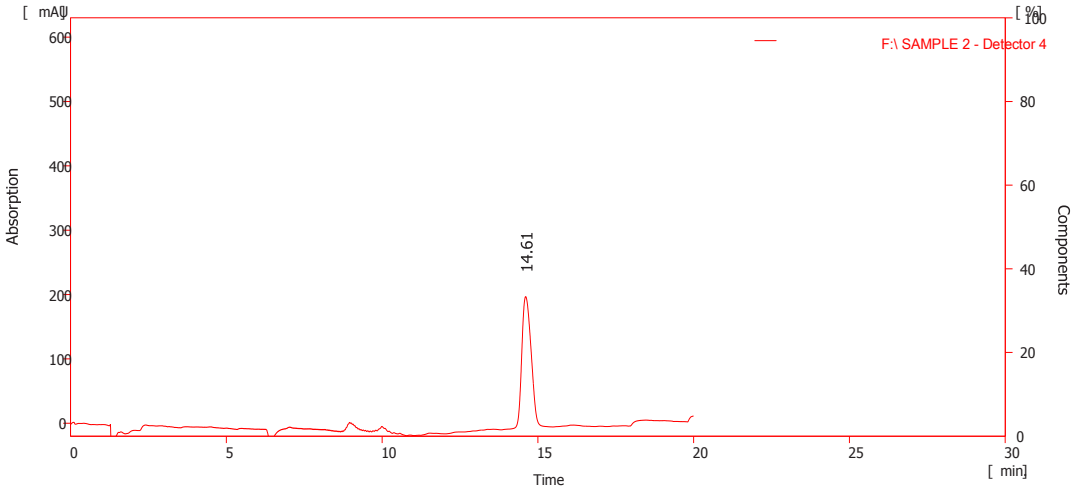

Fig. 4b. HPLC analysis of *Laetiporus sulphureus* lovastatin

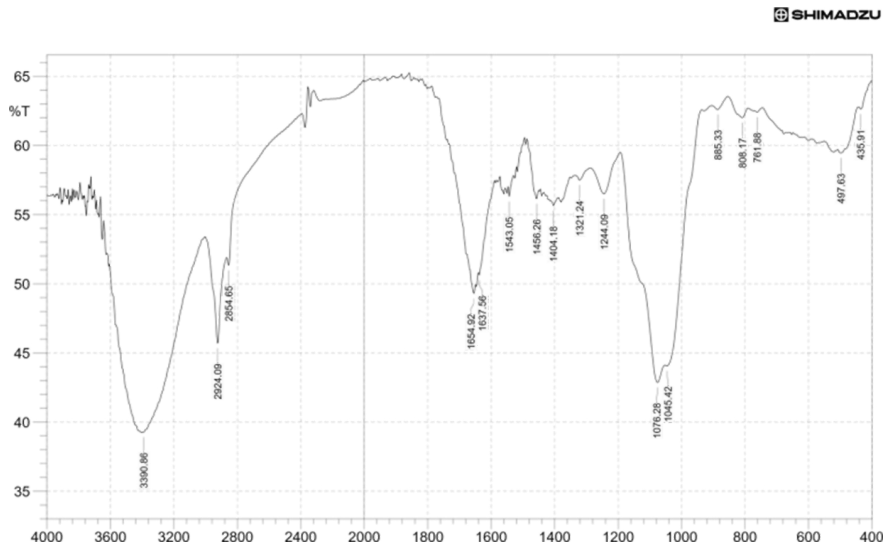

Fig. 5. FTIR for lovastatin extracted from *Laetiporus sulphureus*

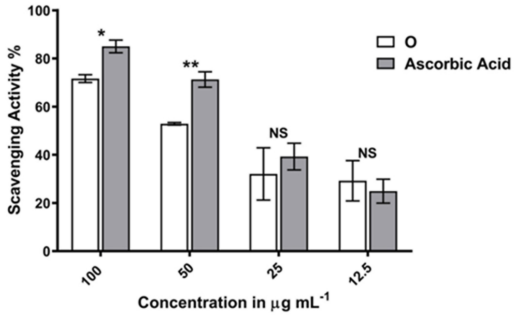

Fig. 6. The antioxidant efficacy of lovastatin extracted from *Laetiporus sulphureus*

References

Ajith, T., Harikumar, K., Thasana, H., Sabu, M., Babitha, N. (2006) Proapoptotic and antitumor activities of the HMG-CoA reductase inhibitor, lovastatin, against daltons lymphoma ascites tumor in mice. *Clinica Chimica Acta*, **366**(1-2), 322-8.

Atli, B., Yamac, M. (2012) Screening of medicinal higher basidiomycetes mushrooms from Turkey for lovastatin production. *International Journal of Medicinal Mushrooms*, **14**, 149-159.

Bhargavi, S., Praveen, V.K., Marium, S., Sreepriya, M., Savitha, J. (2016) Purification of lovastatin from *Aspergillus terreus* (KM017963) and evaluation of its antioxidant properties. *Asian Pacific Journal of Cancer Prevention*, **17**(8), 3795-3801.

Bhuvaneshwari, R.S., Anand, P.G. (2018) Physicochchemical characterization and dissolution study of spray dried amorphous lovastatin with polyvinylpyrrolidone K30. *The Pharma Innovation Journal*, **7**(3), 498-502.

Dhar, R., Choudhury, G.B., Nigam, V.K. (2015) Screeninig of different fungi for production of lovastatin. *Asian Journal of Biomedical and Pharmaceutical Sciencess*, **5**(44), 24-29.

Goswami, S., Bhunia, B., Mandal, T. (2013) Purification and characterization of lovastatin from *Aspergillus terreus* (JXO81272.1). *Journal of Pharmascitech*, **3**(1), 27-30.

Jaivel, N., Narimuthu, P. (2010) Isolation and screening of lovastatin producing microorganisms. *International Journal of Engineering Science and Technology*, **2**(7), 2607-2611.

Janani, B., Saibaba, G., Archunan, G., Vidhya, K., Karunyadevi, J., Angayarkanii, J. (2017) Purification and cytotoxicity study of lovastatin from soil fungi. *Asean Journal of Pharmaceutical and Clinical Research*, **10**(10), 258-262.

Kala, K., Poprawa, A.K., Rzewinska, A., Muszynska, B. (2020) Fruiting bodies of selected edible mushrooms as a potential source of lovastatin. *European Food Research Technology*, **246**(22), 713-722.

Khalil, N., Yousef, R. (2020) Efficacy of *Alternaria alternata* NMK1 secondary metabolites against some seed-borne fungi', *Egyptian Journal of Botany,* **60**(2), 537-548.

Lung, M.Y., Huang, W.Z. (2012) Antioxidant properties of polysaccharides from *Laetiporus sulphureus* in submerged cultures. *African journal of Biotechnology*, **11**(23), 6351-6358.

Mangunwardoyo, W., Rafliyant, Y., Kusmana, D. (2012) Bioprospect of lovastatin in *Aspergillus* spp. from universityof Indonesia (UICC). *World Applied Sciences Journal*, **16**(2), 183-188.

Mohan, K.H., Dhale, M.A., Naidu, K.A., Vijayalakshmi, G. (2011) Antioxidant effect of red mould rice in hyper cholesterolemic Wistar male rats. *Cell Biochemistry and Function*, **29**(7), 597-602.

Neduri, K., Bontha, V.K., Vemula, S.K. (2012) Different techniques to enhance the dissolution rate of lovastatin: Formulation and evaluation. *Asian Journal of Pharmaceutical and Clinical Research*, **6**(1), 56-60.

Nidhiya, A., Sebastian, J.E.S., Raman, N.M., Ramasamy, S. (2013) Extraction and purification of lovastatin from non-aflatoxigenic strains of *Aspergillus flavus*. *International Journal of Biological and pharmacutical Research*, **4**(12), 916-921.

Pandey, V.V., Varshney, V.K., Pandey, A. (2019) Lovastatin: A journey from ascomycetes to basidiomycetes fungi. *Journal of Biologically Active Products from Nature*, **9**(3), 162-178.

Praveen, V., Santebennur, B., Savitha, J. (2014) Endophytic fungi: A poor candidate for the production of lovastatin. *British Microbiology Research Journal*, **4**(12), 1512-1520.

Pushpa, H., Priyata, H., Nomita, D. K., Onya, N., Vijayalakshmi, A., Ramesh, D. (2016) Screening of lovastatin (HMG-CoA reductase inhibitor) from edible wild mushrooms. *Current Research in Environmental and Applied Mycology*, **6**(3), 190-196.

Puttananjaiah, M.K.H., Dhale, M.A., Gaonkar, V., Keni, S. (2011) S:3-Hydroxy-3methylglutaryl-CoA(HMG-CoA) reductase inhibitors demonstrate anti-atherosclerotic character due to their antioxidant capacity. *Applied Biochemistry and Biotechnology*, **163**(2), 222-215.

Raj, R., Gupta, S.K., Verma, M. (2019) Microbial fermentation of lovastatin and other bioactive seconary metabolites using *Aspergillus terreus*. *Research Journal of Life Sciences, Bioinformaticcs, Pharmaceutical and Chemical Sciences*, **5**(3), 34-46.

Raman, R., Krishna, R., Muthusamy, P. (2012) Production and cytotoxicity studied of lovastatin from Aspergillus niger PN2 an endophytic fungi isolated from *Taxus baccata*. *International Journal of Applied Biology and Pharmaceutical Technology*, **3**(3), 342-351.

Rengarajan, S., Sankaranarayanan, S., Bana, P., Ramachandran, J., Vijayalakshmi, M., Deecaraman, M. (2013) Antioxidant and antihemolytic of flavonoid extract from fruit peel of *Punica granatum*. *Asian Journal of Pharmaceutical and Clinical Research*, **6**(2), 208-211.

Seenivasan, A., Panda, T., Gummadi, S., Theodore, T., Sathyansarayana, N. (2015) Quantification of lovastatin produced by *Monascus purpureus*. *The Open Biotechnology Journal*, **9**(1), 6-13.

Shivakumar, S., Ravurim, M. (2018) Lovastatin production from endophytic *Meyerozyma guilliermondii* isolated from *Hibiscus rosa-sinensis*. *International Journal of Biology,Pharmacy and Allied Seinces(IJBPAS)*, **7**(8), 1454-1463.

Talkad, M.S., Das, R.K., Bhattacharjee, P., Ghosh, S., Shivajirao, U.P. (2015) Establishment of enzyme inhibitory activities of lovastatin, isolated from pleurotus ostreatus. *International Journal of Applied sciences and biotechnology*, **3**(3), 408-416.

Vilches feron, M.A., Casas lopez, J.L., Sanchez perez, J.A., Fernandez sevilla, J.M., Chisti, Y. (2005) Rapid screening of *Aspergillus terreus* mutants for overproduction of lovastatin. *World Journal of Microbiology and Biotechnology*, **21**(2), 123-125.

استخلاص وتنقية Lovastatin من الفطر *Laetiporus sulphureus* وتحديد فعاليته المضادة للاكسدة

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اللوفاستاتين من العقاقير التي تعمل على خفض مستوى الكولسترول في الدم، ينتج من الأيض الثانوي للعديد من الفطريات، وفي هذه الدراسة استخلص من الفطر البازيدي *Laetiporus sulphureus* باستخدام المذيب العضوي Ethyle acetate بنظام التخمرات المغمورة. أجري الكشف الأولى عن إنتاج اللوفاستاتين في المستخلص الخام بطريقة الأنتشار بالحفر وتبين له فعالية تثبيطية ضد خميرة *Candida albicans*. استخدمت تقنية كرماتوكرافيا الطبقة الرقيقة TLC للتحري عن اللوفاستاتين وأسفرت عملية الترحيل عن ظهور بقعة متميزة بقيمة RF مقدارها 0.55 مماثلة للوفاستاتين القياسي. ولمعرفة مدى نقاوة الوفاستاتين أستخدمت تقنية كروماتوكرافيا السائل عالي الأداء HPLC ظهرت حزمة امتصاص واحدة بتقنية HPLC عند زمن احتجاز مقداره 14.64 دقيقة. واشارت نتيجة الكشف عن المجاميع الفعالة في اللوفاستاتين المنقى بطيف الاشعة تحت الحمراء FTIR Spectrum إلى وجود مجاميع الامايد والكاربوكسيل كما وأظهر اللوفستاتين المنقى فعالية بايولوجية مضادة للأكسدة بأختبار DPPH أذ بلغت الفعالية التثبيطية 75% عند التركيز 100 مايكروغرام/ مل مقارنة مع مضاد الأكسدة القياسي Ascorbic acid التي بلغت 85%.