IMMOBILIZATION AND APPLICATION OF PARTIAL PURIFIED LOVASTATIN PRODUCED FROM LOCAL ISOLATE *ASPERGILLUS TERREUS* A50 USING SOLID STATE FERMENTATION

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ABSTRACT

This work was designed to study the free and immobilized partial purified lovastatin in various applications. The results of HMG-CoA reductase inhibition showed enzyme inhibition at 10 mM of standard and partial purified lovastatin with specific activity 0.056 and 0.062 U/mg protein respectively, compared with specific activity 0.277 U/mg protein without inhibitor. The results of the thermal stability and storage time on lovastatin for inhibition of HMG-CoA reductase demonstrated that the standard and partial purified lovastatin were stabled in temperatures between 20-40 °C, then the stability begun to decrease at 45 °C, while lovastatin was stable in storage time between 1- 8 hours, then the stability begun to decrease after ten hours at 40 °C. The results of MIC for lovastatin were demonstrated that most tested concentration were showed antibacterial activity of free and immobilized partial purified lovastatin against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* with MIC values ranging from 15 to 75 µg/ml. Whereas the results of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) showed that *C. albicans*, *E. coli*, and *S. aureus* had no growth with concentration ranging from 55 to 75, 55 to 75, and 30 to 75 µg/ml, respectively. As well as the results of the cytotoxic impact using MTT experiment indicated that partial purified lovastatin caused a reduction in cells viability ($p \leq 0.05$) at a dose-dependent manner on MCF-7 cell lines, with a calculating IC_{50} of 138.1 µg/ml, compare with normal cell line (WRL 68 Cell Line) at IC_{50} of 198.7 µg/ml.

Keyword: silver nanoparticles, HMG-CoA reductase activity, antimicrobial activity, anticancer activity.

تقييد وتطبيق اللوفاستاتين المنقى جزئياً والمنتج من العزله المحلية لفطر *Aspergillus terreus* A50 باستخدام تخمرات الحالة الصلبة

علي جبار رشك الساعدي **غازي منعم عزيز**
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المستخلص

صمم العمل الحالي لدراسة اللوفاستاتين المنقى جزئياً الحر والمقيد في مختلف التطبيقات. أظهرت نتائج تثبيط انزيم HMG–CoA Reductase أن الإنزيم ثبط عند 10 ملي مولر من اللوفاستاتين القياسي والمنقى جزئياً مع فعالية نوعية 0,056 و 0,062 وحدة/ملغرام بروتين على التوالي ، مقارنة مع الفعالية النوعية للانزيم 0,277 وحدة/ ملغرام بروتين وبدون مثبط. كما أظهرت نتائج الثبات الحراري وتأثير اوقات التخزين المختلفة على اللوفاستاتين لتثبيط انزيم HMG–CoA Reductase أن اللوفاستاتين المنقى جزئياً والقياسي كان مستقر في درجات حرارة تتراوح بين 20–40 درجة مئوية ، ثم بدأ الثبات ينخفض عند 45 درجة مئوية ، بينما كان تأثير وقت التخزين المتنوع على اللوفاستاتين مستقرا في وقت التخزين بين 1–8 ساعات ، ثم بدأ الثبات ينخفض بعد عشر ساعات عند 40 درجة مئوية. أظهرت نتائج الحد الأدنى من التركيز المثبط (MIC) للوفاستاتين أن معظم التراكيز التي تم اختبارها أظهرت فعالية مضاده للمايكروبات باستخدام اللوفاستاتين المنقى جزئياً الحر والمقيد ضد خميرة *Candida albicans* ، و *Escherichia coli* و *Staphylococcus aureus* مع قيم MIC تتراوح من 15 إلى 75 ميكروغرام/ مليلتر. بينما أظهرت نتائج الحد الأدنى من التركيز القاتل للبكتريا (MBC) والحد الأدنى من التركيز القاتل للفطريات (MFC) أن *C.albicans* و *E.coli* و *S.aureus* لم تظهر اي نمو في التراكيز التي تتراوح من 55 إلى 75 , و 55 إلى 75 , و 30 إلى 75 ميكروغرام/مليلتر، على التوالي. أشارت نتائج التأثير السام على الخلايا باستخدام تجربة MTT إلى أن اللوفاستاتين المنقى جزئياً تسبب في انخفاض حيوية الخلايا ($p \leq 0.05$) بطريقة تعتمد على الجرعة وعلى خطوط خلايا MCF–7 ، مع حساب IC50 حوالي 138,1 ميكروغرام/ مليلتر، مقارنة مع خط الخلية العادي (خط الخلية WRL 68) عند IC50 حوالي 198,7 ميكروغرام/مليلتر.

الكلمات المفتاحية: جزيئات الفضة النانوية، انزيم HMG–CoA Reductase , الفعالية المضادة للميكروبات، الفعالية المضادة للسرطان.

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INTRODUCTION

Lovastatin is polyketide components, created or produce via certain fungi during their secondary metabolism. Lovastatin, also known as 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) inhibitors, are a class of lipid-lowering medications that reduce illness and mortality in those who are at high risk of cardiovascular disease. The statins act as competitive inhibitors for HMG-CoA reductase, a rate constraining advance of cholesterol biosynthesis (2). Statins hinder the change of HMG-CoA to mevalonic acid in the mevalonate pathway (18). Production of lovastatin by submerged fermentation and solid state fermentation has been widely investigated and commonly, filamentous fungi exhibit tremendous potentiality (7). *Aspergillus terreus* is a filamentous ascomycota, which is significant provenance for generation of lovastatin (6). The immobilization methods are used for the binding of cells, organelles, compounds, proteins (3), or different materials onto a strong help, into a strong lattice or held by a membrane, so as to build their stability and make conceivable their rehashed or proceeded with employ (16). Silver particles having fine or ultrafine sizes have attracted scientific interest because of their unusual properties compared to bulk metal (19). Colloidal particles because of their quantum size effects and surface effects reveal excellent electrical conductivity, catalytical activity, chemical stability and antimicrobial activity (14). Interestingly, some studies have demonstrated an antimicrobial potential for statins against different bacterial species. For example, simvastatin was able to inhibit host-cell invasion and *Staphylococcus aureus* growth. In addition, lovastatin, atorvastatin, simvastatin and rosuvastatin showed activity against several reference bacteria, yeasts and clinical isolates (11). Lovastatin and associated components indicated wonder impacts on tumor cells however site of activity and mechanism of activity is ineffectively comprehended. Concentrate done via Xiangli *et.al.* uncovered anti-proliferative impacts of lovastatin on malignant cells. The experiment was performed on the tumor in human glioblastoma cells and decrease in the malignancy was showed by lovastatin through inactivation of RAS farsonylation (29). The purpose of this study was to immobilization of lovastatin produced from local isolate *Aspergillus terreus* A50 using solid state fermentation with nano-silver and used of lovastatin in various applications.

MATERIALS AND METHODS

Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Nutrient agar, Nutrient broth from Hi-media, India. HMG-CoA Reductase activity kit (colorimetric) from abcam company. Sodium Acetate (CH3COONa), Sodium Hydroxide (NaOH), Ethanol 95%, methanol, ethyl acetate, trifluroacetic acid and other materials from BDH, England.

Lovastatin production

Collection of seventy three local fungal isolates from different areas, and screening for lovastatin production. The local isolate *Aspergillus terreus* A50 was best isolate for lovastatin production through submerged fermentation. The optimum conditions for lovastatin production by local isolate *A.terreus* A50 were used solid state fermentation (SSF) with media contain wheat bran and oat bran (1:1 w:w), sodium acetate, moisture ratio (1.2:1 v:w), pH (7), incubation temperature 30 °C and incubation period (6 days). Local isolate of *A.terreus* A50 was cultured on potato dextrose agar. Then lovastatin production from this isolate were performed by using a medium mention above and the growth was elicited with 1 ml ($1x10^6$ cells/ml)/5gm media of *S.cerevisiae* after 48 hours of culture.

Extraction of lovastatin

After the end of SSF operation, the concentration of lovastatin was measured. First, the culture was extracted in 250 ml Erlenmeyer's flask with ethyl acetate (pH 3.0). The mixtures was then incubated in rotating shaker with 140 rpm at 28 ºC for 2 h. Next, filtration was done using Whatman filter paper (No. 1) for separation of the biomass from the filtrate. Then stored the supernatant in glass bottles at 4 ºC until use for additional examination as crude extraction (26).

Estimation and purification of lovastatin

Lovastatin was estimated based on the method described by Mielcarek *et al.* (21) as follows: One ml of trifluroacetic acid (1%) was mixed with one ml of the supernatant and incubated for 10 min. A 0.5 ml from these mixture was diluted 10 times with methanol and its absorb-

ance was estimated at 238 nm using UV-Visible Spectrophotometer. The lovastatin concentration in the sample was determined by plotting the O.D values on standard chart. Three ml of mixture from 0.5 ml trifluroacetic acid (1%) and 2.5 ml methanol were used as control. The purification of lovastatin produced by local isolate *A.terreus* A50 was performed by silica gel column chromatography (using silica gel column (67-1.5 cm) (60-120 Mesh) eluted with chloromethane :ethyl acetate (70:30 v:v), 3 ml for each fraction with flow rate 20 ml/ hour).

Effects of partial purified lovastatin on HMG-CoA Reductase activity

The study of the partial purified lovastatin effect on HMG-CoA reductase activity was performed by using kit (colorimetric) from abcam company. This experiment was achieved using 10 mM of standard, partial purified lovastatin, and atorvastatin (present in kit as standard inhibitor) . The enzyme was separately incubated with each inhibitor at 37 ºC each 2 min for 10 min. The the percentage (%) of enzyme inhibition was estimated according to the producer of kit (colorimetric) from abcam company. The enzyme inhibition was estimated according to the equation:

Inhibition %=[(−Δ**A 340nm/ΔT** (**Enzyme**)) – (−Δ**A 340nm/ΔT** (**Enzyme**+**Inhibitor**)] / −Δ**A 340nm/ ΔT** (**Enzyme**) }X 100.

A= Absorbance, T= Time

Thermal stability of lovastatin for HMG-CoA reductase inhibition: A 100 µl of standard and partial purified lovastatin separately were incubated in water bath at different temperature degrees (20, 25, 30, 35, 40, 45, 50, 55, and 60) ^{O}C for 30 min, then the test tubes containing the lovastatin were transferred directly to cold water. The impacts of inhibitors on HMG-CoA Reductase activity were determined by using 5 µl (10 mM) of standard and partial purified lovastatin. The enzyme was separately incubated with inhibitors at 37 ºC each 2 min for 10 min. The enzyme inhibition % was estimated according to the same previous equation.

Effect of different storage time of partial purified lovastatin on HMG-CoA reductase inhibition: A 100 µl of standard and partial purified lovastatin separately were incubated in water bath at 40 ^{O}C for different time 2, 4, 6, 8, 10, 24, 48 hours. The test tubes containing the lovastatin were then transferred directly to cold water bath. The effects of inhibitors on HMG-CoA Reductase activity were investigated by using 5 µl (10 mM) of standard and partial purified lovastatin. The enzyme was separately incubated with inhibitors at 37 ºC each 2 min for 10 min. The enzyme inhibition % was estimated according to the same previous equation.

Immobilization of lovastatin (**Binding Nanosilver with lovastatin)**: Lovastatin was suspended in deionizing water and sonicated for 15 min. Next, 2 ml of lovastatin suspension (0.5 mg/ml) was transferred to tube and mixed with 2 ml of standard silver nanoparticles of concentration 0.7 mg/ml. After incubation for 24 hours the solutions were centrifuged 10000 rpm, for 10 minutes at room temperature, the supernatant was transferred to tube and ignores. While the precipitate was washed for four times, then were subjected to complete analysis via ultraviolet-visible spectrophotometry and ATR-FTIR analysis, for detect the binding between lovastatin and standard silver nanoparticles (28).

Lovastatin applications

Antimicrobial activity of lovastatin: The bacterial isolates were obtained from department of Biotechnology/College of Science/ University of Baghdad, bacteria were: Gram-positive: *Staphylococcus aureus* and *Enterococcus faecalis*, Gram-negative: *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* and the yeast: *Candida albicans.* The bacteria and yeast were sub-cultured on nutrient broth and incubated at 37°C, while yeast in 28 ºC for 48 hours and then kept in the refrigerator for next experiments. The various concentrations of partial purified lovastatin were prepared using distilled water as solvent, concentration included 10, 50, and 75 µg/ml. The antibiotic (metronidazole) was prepared via distilled water with 50 µg/ml concentration. The metronidazole was used as control (1).

Determination of lovastatin activity by agar diffusion method: According to Obeidat *et al*. (23), petri-dish plates contained of Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for *C.albicans* were used. Agar media were cultured with an overnight culture of

indicators strains. Five wells (5 mm diameter) were made into the (two plates) agar via cork-borer and three wells were loading with 0.1 ml of the partial purified lovastatin with different concentration (10, 50, and 75 µg/ml) and standard lovastatin with concentration of 75 µg/ml, as well as the metronidazole and distilled water were used as control. The inoculums volume was balanced in order to convey last inoculums of around 7×10^6 cells/ml, cells enumeration was doing by a hemocytometer. Incubation of bacteria were performed at 37 ºC and of yeast at 28 ºC for 24 hours. The assessment of antibacterial and antifungal activity was based on diameter measurement of the inhibition zone formed around the well.

Antimicrobial activity of immobilized lovastatin: Petri-dish plates of Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for *C.albicans* were used. Agar media were cultured with old culture (7×10^6 cells/ml) of same microbial strains that mention above. Five wells (5 mm diameter) were made into (two plates) the agar via cork-borer and 0.1 ml of different solutions were applied in each well, these solution including the 75 µg/ml of standard lovastatin, partial purified lovastatin, immobilized lovastatin, nano-silver, and distilled water as control. The assessment of antibacterial and antifungal activity was based on diameter measurement of the inhibition zone formed around the well (23).

Determination of minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC): When in doubt, a concentrate is viewed as dynamic against the two fungi and bacteria if the inhibition zone was more noteworthy than 6 mm (22). Fifteen concentrations for the solutions were prepared (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75 µg/ml)**,** these solution including nano-silver, partial purified lovastatin, immobilized lovastatin, and distilled water as control. Petri-dish plates consist of Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for *C.albicans* were used. Agar media were cultured with old culture (7×10^6 cells/ml) of the microbial strains, including Gram-positive: *S.aureus*, Gram-negative: *E.coli* and the yeast: *C.albicans*. Three wells (5 mm diameter) were performed and 0.1 ml of different concentration solutions were applied in each well. The results were observed and measuring the diameter of inhibition zones (clear zone without growth around the colony) (10). The determination of MBC and MFC was performed by sub-culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar were transferred respectively into plates containing nutrient agar for bacteria and Sabouraud dextrose agar for *Candida albicans*, then incubated at 37 and 28 °C for bacteria and yeast, respectively, for 24 hours and were observed for growth. If there was no growth, the solutions were identified as bactericidal or fungicidal (24).

The cytotoxic effect of partial purified lovastatin: The strategy of this experiment (*in vitro*) was performed to examine the conceivable cytotoxic impact of standard and partial purified lovastatin on tumor cell lines (Michigan cancer foundation-7, MCF-7) and normal cell line WRL 68 (human liver cell line). The cytotoxic effect study was according to the manufacturer's instructions (27):

The cells (1×10^4 to 1×10^6 cells/ml) were cultured in 96-well plates to end volume of 200 µl complete culture medium for each well. The plates were wrapped with a sterile parafilm, gently stirred and incubated for 24 hours at 37 ° C with 5% CO_2. After the incubation, remove of the medium, and 200 µl of a 2- fold various dilution of the standard and partial purified lovastatin (6.25, 12.5, 25, 50, 100, 200, 400 µg/ml) was added to the wells. Triplicate was performed at each concentration and control. Then incubation of the plates for 48 hours at 37°C with 5% CO_2. Then, 10 µl of MTT solution was added to each well. The plates were incubated for 4 hours with 5 % CO_2 at 37 °C. The medium was then neatly segregated, and in each well 100 µl of DMSO solution was added then incubated for 5 minutes. Absorbance was measured using an ELISA reader at a wavelength of 575 nm. Statistical analysis was performed on the optical density readings to calculate the IC50. According to the following equation:

Viability (%) = (Optical density of sample / Optical density of control (live cells)) × 100 %.

RESULTS AND DISCUSSION

Impact of lovastatin on HMG-CoA Reductase activity: The effects of inhibitors on HMG-CoA Reductase activity were determined by using 10 mM of standard and partial purified lovastatin. The results of this experiment show that HMG-CoA Reductase activity was inhibited at 10 mM of standard and partial purified lovastatin with specific activity 0.056 and 0.062 U/mg protein respectively, compared with control (Figure 1), while (10 mM) atorvastatin (in kit as standard inhibitor) inhibit the HMG-CoA Reductase activity with specific activity 0.039 U/mg protein.

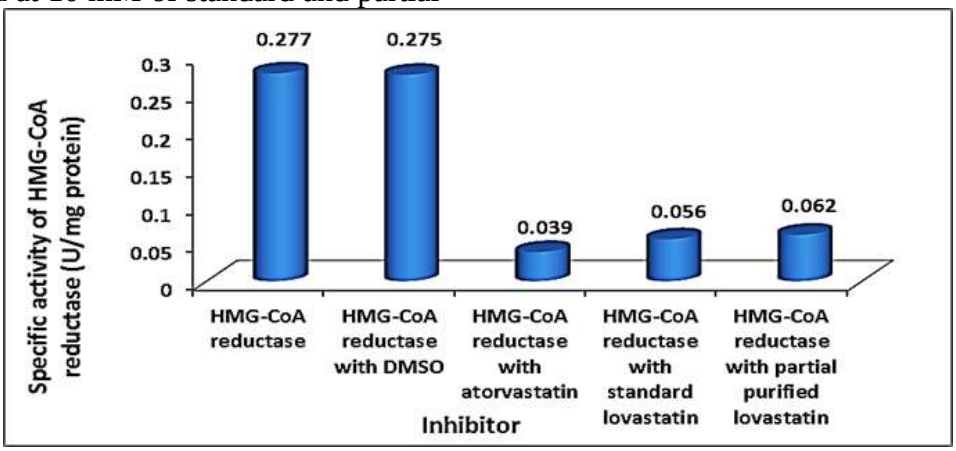


Figure 1. Effect of standard and partial purified lovastatin on HMG-CoA reductase activity

Peter and Jones (25), found that the statins have proved to be potent therapies for reducing elevated low-density lipoprotein (LDL) cholesterol and lessening the risk of coronary heart disease (CHD) and related events via inhibiting HMG-CoA Reductase enzyme. Zipp**,** *et.al.* (30), proved that brain cholesterol levels in mice were reduced after treatment with 100 mg/kg of lovastatin, no reduction was observed in guinea pigs treated with much higher doses of simvastatin over the same period of time, duo to the lovastatin is more effect on of HMG-CoA Reductase enzyme than simvastatin.

Thermal stability of lovastatin for inhibition of HMG-CoA reductase activity: The results of this study demonstrated that the standard and partial purified lovastatin were stable in temperatures between 20–40 °C, then the stability begun to decrease at 45 °C (Figure 2). This decrease in lovastatin may be due to the thermal effect on the lovastatin activity and/or structure.

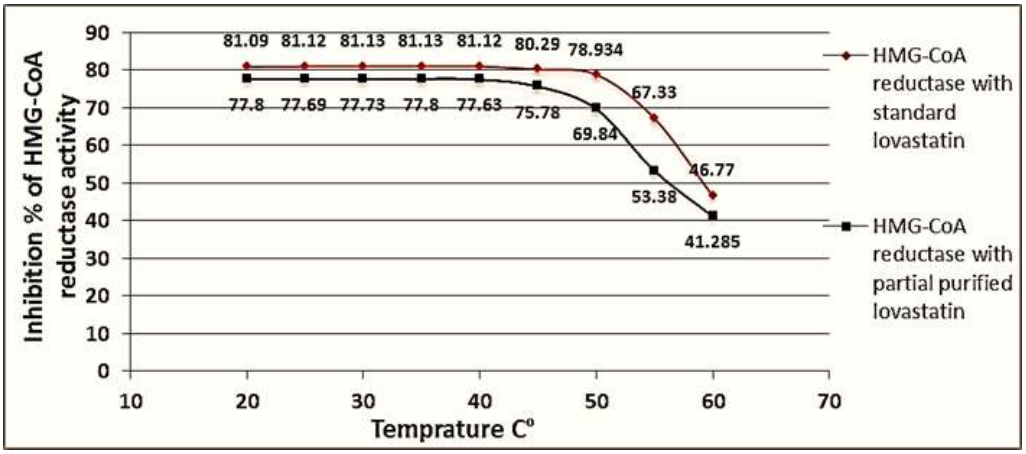


Figure 2. Effect of different temperature on inhibitors (standard and partial purified lovastatin) for of HMG-CoA reductase inhibition

The literature mention that the storage of lovastatin must be at controlled room temperature 20-25 °C, and must avoid excessive heat and humidity. Ho *et.al*. (13), demonstrated that lovastatin exhibits higher thermal stability and lower degradation rate than simvastatin. Candyrine *et.al.* (9), showed that the best temperature for lovastatin storage was from 22 to 25 °C.

Lovastatin stability at different storage time

The results of this study showed that the standard and partial purified lovastatin were

stable in storage time between 1–8 hours, then the stability begun to decrease after the ten hours (Figure 3).This decreases in inhibition by lovastatin may be due to the thermal effect on the lovastatin structure. Some literature reported that the temperature of lovastatin storage was at 20-25°C, also lovastatin must be protected from light and stored in a well-closed, light-resistant container (9).

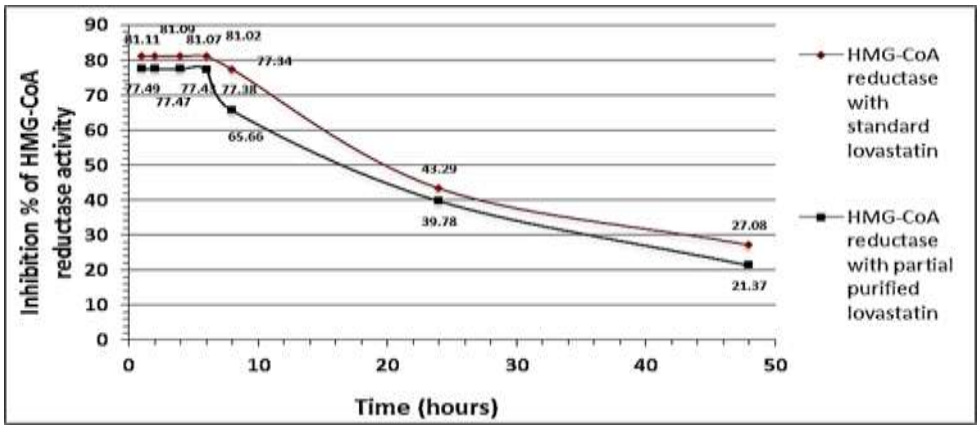
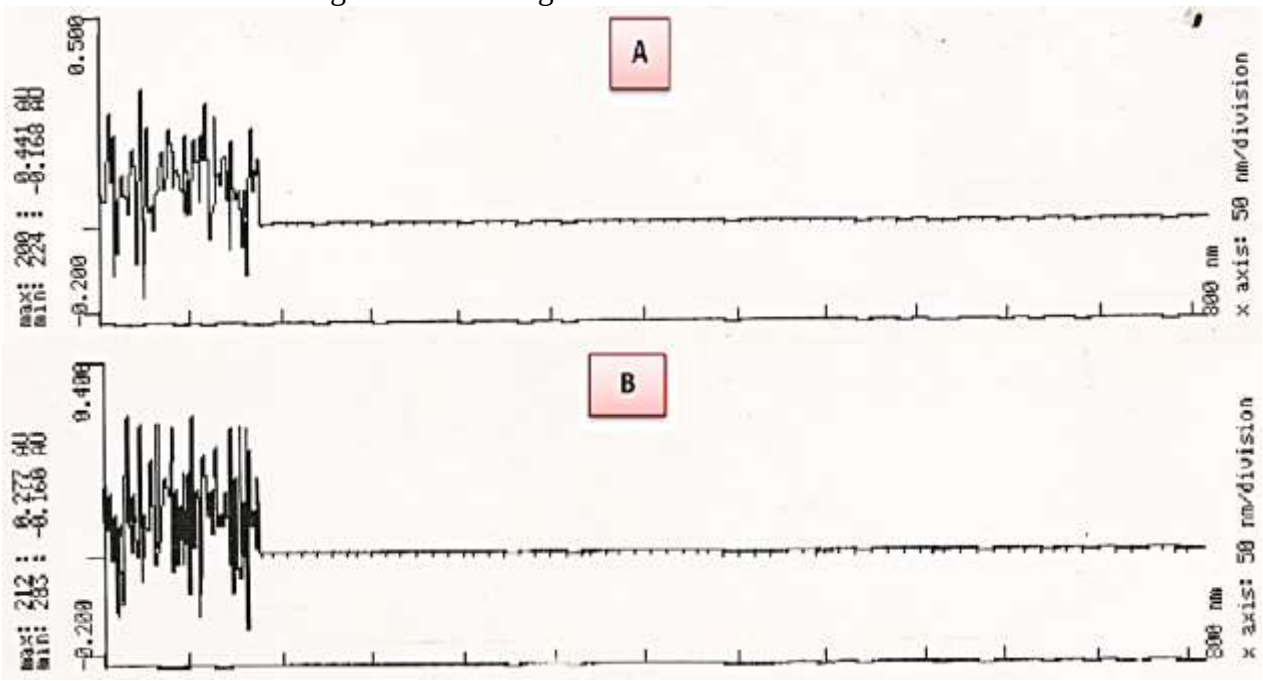


Figure 3. Effect of different storage time on lovastatin for HMG-CoA reductase inhibition

Immobilized lovastatin (Nanosilver-lovastatin): The results showed that the nano-silver was bound with partial purified lovastatin to form immobilized lovastatin. Whereas the maximum absorbance of free standard nano-silver, free partial purified lovastatin, and standard nano-silver plus partial purified lovastatin without binding after scanning at 200-800 nm, were 200, 212, and 228 nm, respectively (Figure 4-A,B,C). While the maximum absorbance of immobilized lovastatin was 288 nm, this indicate that the lovastatin was binding with nano-silver to form one large molecule called immobilized lovastatin (figure 4-D).



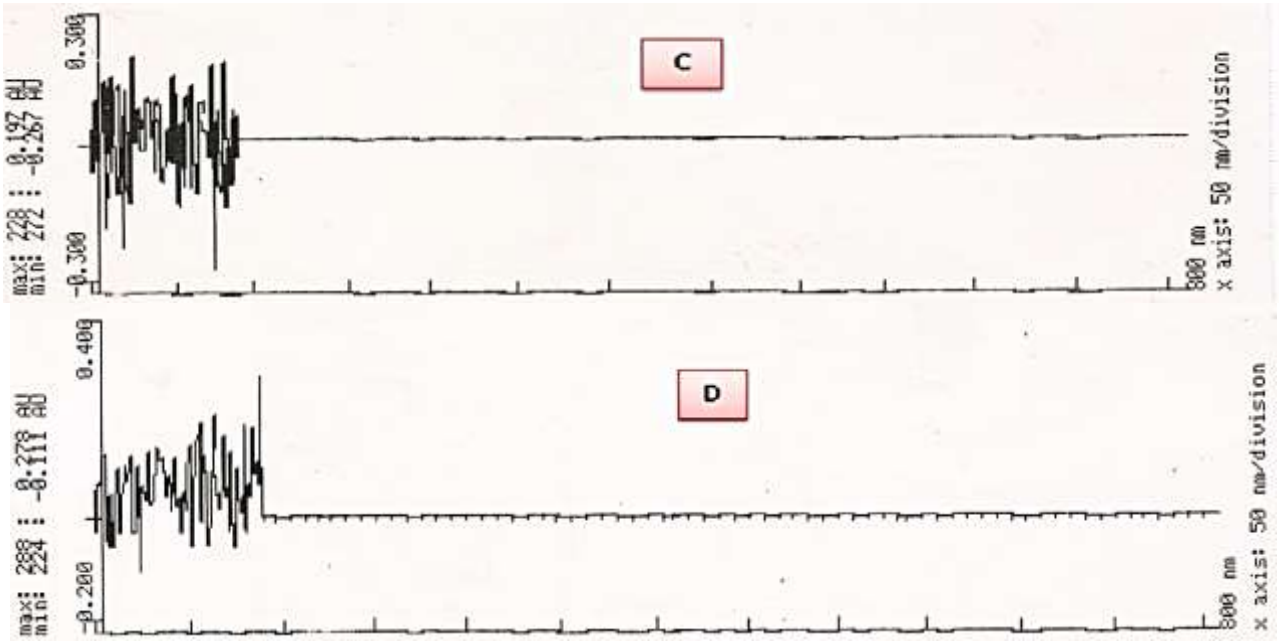


Figure 4. Scanning of absorbance for Nano silver and lovastatin at 200-800 nm: A- Standard Nano-silver; B- Free partial purified lovastatin; C- Standard nano-silver and partial purified lovastatin mixture without binding; D- Immobilized lovastatin

According to the outcomes in Figures (5 and 6) and compared with ATR-FTIR analysis of partial purified lovastatin, the FTIR spectra of immobilized lovastatin shows that the peaks at 1616.24 cm^{-1} for lovastatin-silver nanoparticles is due to alkenes C=C group, that has been shifted to a higher wavenumber in comparison to the primary aromatic C=C of partial purified lovastatin (1560.30 cm^{-1}), which suggested that the role of primary aromatic C=C group of partial purified lovastatin in reduction of lovastatin-silver nanoparticles. The lactone ring bands and other bands do not show any shift in lovastatin-silver nanoparticles as compared to partial purified lovastatin. Hence it can be deduced that formation of lovastatin-silver nanoparticles and lactone ring of partial purified lovastatin preserves its integrity (figure 5 and 6).

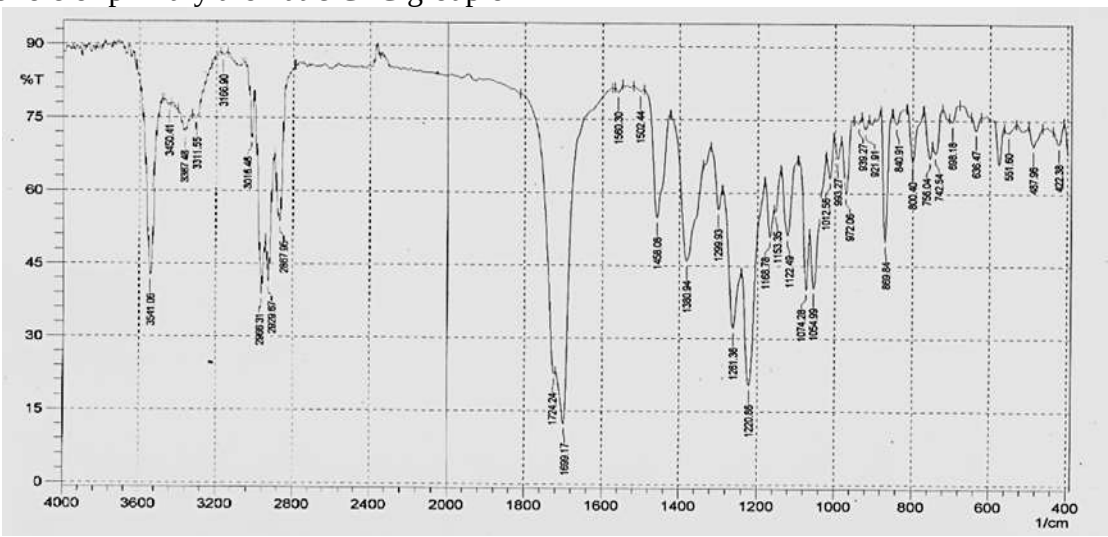


Figure 5. ATR-FTIR analysis of partial purified lovastatin from local isolate *A.terreus* A50 using solid state fermentation

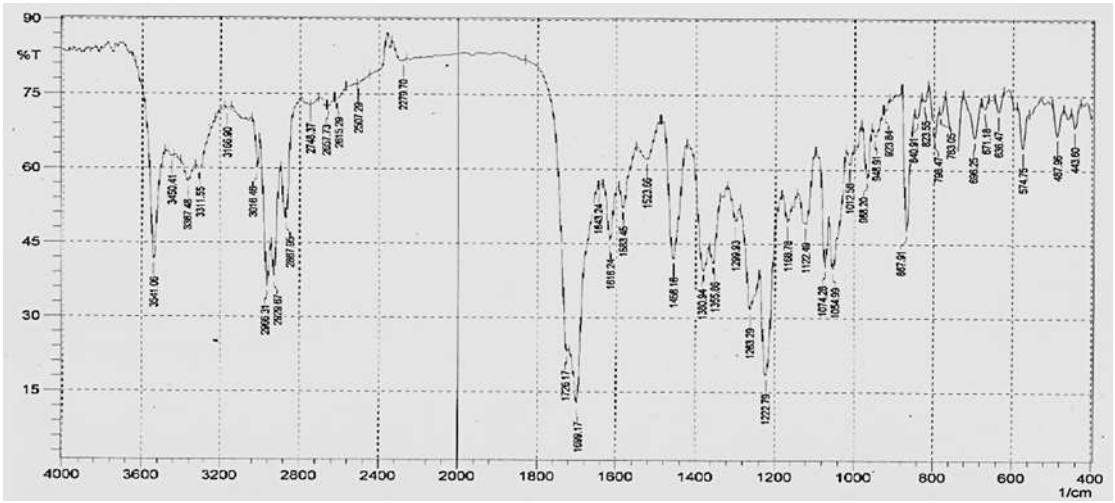


Figure 6. ATR-FTIR analysis of immobilized lovastatin

Antimicrobial activity of lovastatin

The antibacterial and antifungal activity of partial purified lovastatin against fungi and bacteria are presented in Table (1). The isolates were sensitive to partial purified lovastatin. *Staphylococcus aureus* and *S.typhi* more sensitive to partial purified lovastatin than other Gram negative, Gram positive bacteria, and yeast, with inhibition zones of 24 mm and 23 mm respectively, at concentration 75 µg/ml. The inhibition zone of *E. faecalis* was 18 mm while the inhibition zones of each of *E.coli*, *C.albicans*, and *P.aeruginosa* were 22, 20 and 13 mm, respectively, at concentration 75 µg/ml. Also, the other concentrations (10, and 50 µg/ml) of partial purified lovastatin were showed low inhibition zones compare with 75 µg/ml concentration. Base on the results, it can be said that concentrations of partial purified lovastatin (10, 50, and 75 µg/ml) gives antimicrobial activity against both bacterial and fungi isolates (Table 1) according to Muhammad and Muhammad (22).

Table 1. Impact various concentrations of partial purified lovastatin on the microbial isolates

NO.	Isolate	Inhibition zone (mm) of antibiotic (metronidazole) (50 µg/ml) as control	Inhibition zone (mm) of lovastatin 25 µg/ml	Inhibition zone (mm) of lovastatin 50 µg/ml	Inhibition zone (mm) of lovastatin 75 µg/ml	Inhibition zone (mm) of standard lovastatin 75 µg/ml
1	*C. albicans*	28	15	17	20	20
2	*S. aureus*	27	16	19	24	23
3	*E. faecalis*	22	11	15	18	16
4	*E. coli*	26	13	17	22	20
5	*S. typhi*	27	15	18	23	21
6	*P. aeruginosa*	13	8	10	13	12

Masadeh, *et.al*., (20), demonstrated that statins can actuate variable degrees of antibacterial action with atorvastatin, lovastatin and simvastatin being the more strong than rosuvastatin. While vancomycin-defenseless *Enterococci* (VSE), methicillin-sensitive *S.aureus* (MSSA), vancomycin-resistance *Enterococcus* (VRE), methicillin- resistance *S. aureus* (MRSA), *S.epidermidis*, *E.aerogenes*, and *Acinetobacter baumannii*, were progressively sensitive to both simvastatin, atorvastatin, and lovastatin contrasted with rosuvastatin. Then again, *P.mirabilis*, *E.cloacae*, and *E.coli* were increasingly sensitive to atorvastatin contrasted with both lovastatin, simvastatin and rosuvastatin. Moreover, most clinical segregates were less sensitive to statins contrasted with their relating standard strains. Statins are a class of pharmaceutical widely used to treat high serum cholesterol. In addition, statins have so-called pleiotropic impact, which include the reduction of inflammation, immunomodulation, and anti-microbial effects (12).

Antimicrobial activity of immobilized lovastatin: The results in Table 2 show that the tested solutions were effective in inhibiting microbial growth of test microorganisms with different potency. Immobilized lovastatin was more effective against gram negative bacteria than gram positive bacteria and yeast. Immobilized lovastatin possess higher antimicrobial activity when compared with other com-

pounds. The range of inhibition zones for immobilized lovastatin, partial purified lovastatin, Nano-silver were of 31-48, 12-23, 12-17 mm, respectively. The highest antibacterial activity was obtained from immobilized lovastatin against *S. aureus* (48 mm) and the lowest antibacterial activity was against *E. faecalis* (31 mm). The results demonstrated that the *in vitro* antimicrobial activity of immobilized lovastatin were more efficient compared to their respective free forms. In addition, developed a simple technique for the conjugation of lovastatin with nano-silver needs functionalization process. The interaction between lovastatin and nano-silver is likely to be mediated by the adsorption of the lovastatin molecules on the nanoparticle surfaces.

Table 2. Impact of immobilized lovastatin on the microbial isolates used well diffusion test

NO.	Isolate	Inhibition zone (mm) of immobilized lovastatin 75 µg/ml	Inhibition zone (mm) of standard lovastatin 75 µg/ml	Inhibition zone (mm) of partial purified lovastatin 75 µg/ml	Inhibition zone (mm) of Nano-silver 75 µg/ml
1	*C. albicans*	33	19	18	12
2	*S. aureus*	48	23	23	16
3	*E. faecalis*	31	16	18	12
4	*E. coli*	38	20	22	14
5	*S. typhi*	36	19	22	12
6	*P. aeruginosa*	34	13	12	17

Rogowska, *et.al*., (28), found that ampicillin from an unmodified counterpart was less effective against *Klebsiella pneumoniae* and *S.aureus*, and only for *E.coli* is a strong synergistic effect. A similar phenomenon has been observed for amoxicillin-resistant strain *S.aureus*. The concomitant use of silver nanoparticles and amoxicillin in this case, revealed antagonistic effect. It is likely that nanoparticles (Ag) prevent the absorption of a large amount of ampicillin. Nanoparticles bind to the surface of bacterial cells. Brown *et.al*., (8), has shown that ampicillin-resistant bacterial strains such as *P.aeruginosa*, *Enterobacter aerogenes* or *S.aureus* are vulnerable to silver nanoparticles used with this antibiotic. Its performance was even higher than nanoparticles made of silver alone.

Determination of Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Fifteen concentrations for the solutions were prepared (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75 µg/ml), these solutions including nano-silver, partial purified lovastatin, and immobilized lovastatin, as well as distilled water was used as control. Compounds with antimicrobial activity exhibit impacts that kill microorganisms or temporarily reduce their reproduction. Performed the well diffusion method is insufficient to determine the lethal and instrinsic suppression. In addition to the well diffusion test, MIC and MBC/MFC tests are also more effective in determining how an effect is exhibited (4). The minimum inhibitory concentration (MIC) results showed that all tested solutions were showed antibacterial activity against *C. albicans*, *E. coli*, and *S. aureus* with MIC values ranging from 15 to 75 µg/ml. While the results of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) showed that *C. albicans, E. coli*, and *S. aureus* had no growth with concentration ranging from 55 to 75, 55 to 75, and 30 to 75 µg/ml, respectively. The tested solutions showed different levels of antimicrobial activity depending on tested species as shown in table (3).

Table 3. Antimicrobial activity of partial purified and immobilized lovastatin on the bacterial and fungal isolates

NO.	Isolates	Lovastatin conce. µg/ml	MIC (mm)			MBC/MFC
			Immobilized lovastatin	Partial purified lovastatin	Nano-silver particles	
1		75	34	20	14	No growth
2		70	31	19	14	=
3		65	29	19	13	=
4		60	24	18	13	=
5		55	24	17	13	=
6	*C. albicans*	50	23	17	13	Growth
7		45	21	15	13	=
8		40	18	14	12	=
9		35	18	13	12	=
10		30	17	12	12	=
11		25	15	12	12	=
12		20	13	12	11	=
13		15	0	0	0	=
14		10	0	0	0	=
15		5	0	0	0	=
16		75	46	25	20	No growth
17		70	40	24	19	=
18		65	40	22	19	=
19		60	38	21	17	=
20	*S. aureus*	55	36	18	15	=
21		50	34	16	14	=
22		45	34	16	14	=
23		40	32	14	13	=
24		35	30	13	13	=
25		30	29	13	12	=
26		25	26	13	12	Growth
27		20	19	12	0	=
28		15	0	0	0	=
29		10	0	0	0	=
30		5	0	0	0	=
31		75	28	20	16	No growth
32		70	27	19	16	=
33		65	26	18	15	=
34	*E. coli*	60	26	17	15	=
35		55	24	16	13	=
36		50	23	16	13	Growth
37		45	21	15	12	=
38		40	19	13	12	=
39		35	18	11	11	=
40		30	17	11	11	=
41		25	14	11	9	=
42		20	12	9	7	=
43		15	10	8	0	=
44		10	0	0	0	=
45		5	0	0	0	=

Statins have other impacts than lipid decrease, named pleiotropic impacts, for example, mitigating immunomodulatory activities and anti-inflammatory. Numerous examinations have assessed the impact of statins on the counteraction, dismalness and mortality of different irresistible illnesses. A portion of these examinations have demonstrated that statins can forestall the foundation of contaminations or even decrease death rates in patients routinely taking statins. In patients with sepsis and bacteremia, the utilization of statins was related with lower mortality in ongoing examinations. Also, different investigations did not locate the equivalent defensive impact. Curiously, a few investigations have shown an antimicrobial potential for statins against various bacterial species. For instance, simvastatin had the option to restrain have cell attack and *S.aureus* development. Moreover, lovastatin, atorvastatin, simvastatin and rosuvastatin demonstrated action against a few reference microorganisms, yeasts and clinical disconnects (11).

Cytotoxic effect of partial purified lovastatin (*in vitro*): The MTT experiment was achieved to investigate the cytotoxic impact of standard and partial purified lovastatin on tumor cell lines (MCF-7 cell line). This experiment was proceeded to uncover cell viability after Testing a range of concentrations for each component on tumor cell lines. The results presented in Figure (7) show that partial purified lovastatin revealed a decrease to a depress in cell viability in a dose-dependent

manner on MCF-7 cell lines, with a calculating IC_{50} of 138.1 µg/ml compare with normal cell line (WRL 68 Cell Line) at IC_{50} of 198.7 µg/ml. Nevertheless, data the statistical analysis of data demonstrated significant variations ($P \leq 0.0001$) in the pattern of depression among the used concentration in MCF-7 cells and normal cell line regarding the standard and partial purified lovastatin (table 4, 5, 6, and 7). While the results in the table (8 and 9) demonstrated that the comparison between observed and expected responses had no significant differences.

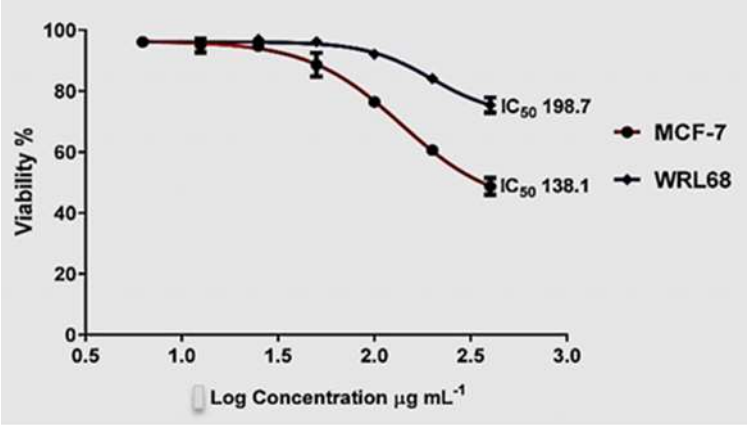


Figure 7. Dose-dependent cytotoxic impact of partial purified lovastatin on MCF-7 cells and normal cell line after incubation at 37°C for 24 hours with 5% CO_2.

Table 4. Convergence information of effect of partial purified lovastatin on MCF-7 cells

	Convergence Information	
	Number of Iterations	Optimal Solution Found
PROBIT	10	Yes

Table 5. Chi-Square Tests of effect of partial purified lovastatin on MCF-7 cells

Chi-Square Tests				
		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	20.726	5	0.001[a]

a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Table 6. Convergence information of effect of standard lovastatin on MCF-7 cells

	Convergence Information	
	Number of Iterations	Optimal Solution Found
PROBIT	10	Yes

Table 7. Chi-Square Tests of effect of standard lovastatin on MCF-7 cells

Chi-Square Tests				
		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	38.790	5	0.000[a]

a. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

Table 8. Cell counts and residuals of effect of partial purified lovastatin on MCF-7 cells

Cell Counts and Residuals							
	Number	concentrations	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	400.000	100	51	59.386	-8.113-	0.594
	2	200.000	100	39	27.383	12.007	0.274
	3	100.000	100	24	15.371	8.163	0.154
	4	50.000	100	11	10.928	.415	0.109
	5	25.000	100	5	9.091	-3.883-	0.091
	6	12.500	100	5	8.263	-3.441-	0.083
	7	6.250	100	4	7.871	-4.013-	0.079

Table 9. Cell Counts and Residuals of effect of standard lovastatin on MCF-7 cells

Cell Counts and Residuals							
	Num-ber	concen-trations	Number of Subjects	Observed Responses	Expected Responses	Residual	Proba-bility
PROBIT	1	400.000	100	60	71.456	-11.194-	0.715
	2	200.000	100	52	33.201	18.767	0.332
	3	100.000	100	29	17.492	11.019	0.175
	4	50.000	100	9	11.797	-2.497-	0.118
	5	25.000	100	4	9.504	-5.222-	0.095
	6	12.500	100	4	8.489	-4.366-	0.085
	7	6.250	100	4	8.013	-3.962-	0.080

Partial purified lovastatin displayed a dose-dependent pattern of piecemeal cytotoxicity starting from the lower concentration to its more strong inhibition at 400µg/ml, 24.575 % inhibition of WRL 68 cells line, and 51.273 % inhibition of MCF-7 cells. The outcomes of cytotoxic impact of partial purified lovastatin indicated that remedying MCF-7 cells at concentrations extending from 6.25 to 400 µg/ml for 24 hours demonstrated an important mortality in cell viability via rising the concentration in a portion subordinate example that came to up to 51.27 % killing at 400 µg/ml with an IC_{50} of 138.1µg/ml (figure 7). In addition, the treatment of MCF-7 cells with standard lovastatin showed an influence by applying the similar concentration range with an identified IC_{50} of 112 µg/mL (figure 8). On the other hand, figure (9 and 10) showed that it is possible to determine the effect of an unknown concentration of standard and partial purified lovastatin by using regression equations according to the following equations:

Y= 1.56+4.65E-3x »for partial purified lovastatin.

Y= 1.56+5.55E-3x »for standard lovastatin

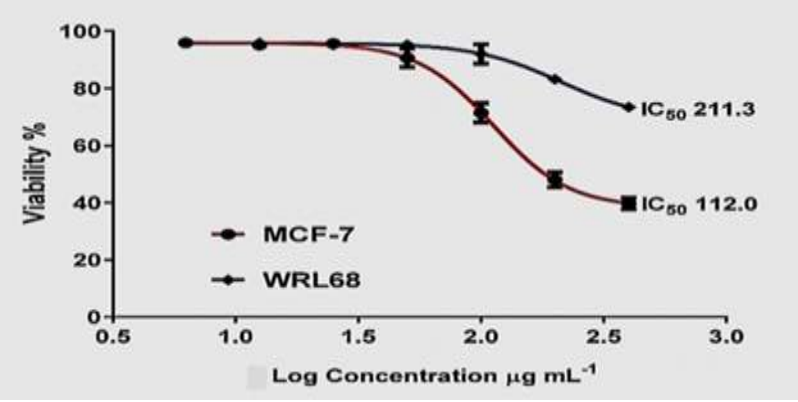


Figure 8. Dose-dependent cytotoxic effect of standard lovastatin on MCF-7 cells and normal cell line after incubation at 37°C for 24 hours

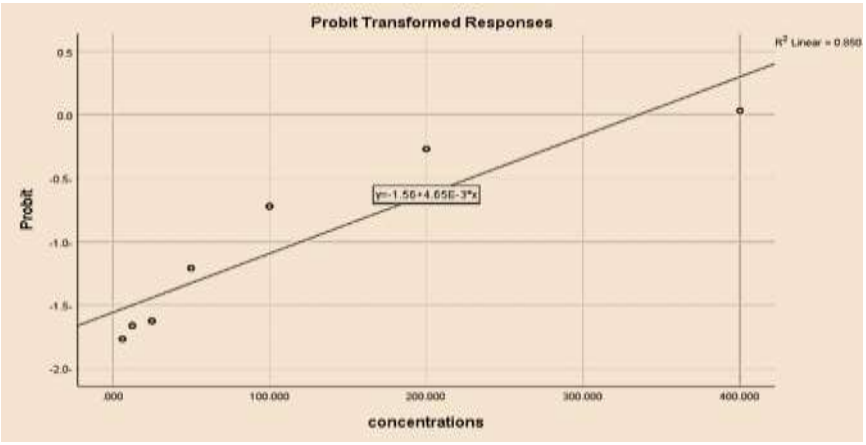


Figure 9. Partial purified lovastatin concentrations with probit inhibition on MCF-7 cells

Figure 10. Standard lovastatin concentrations with probit inhibition on MCF-7 cells

Klawitter *et. al.* (17), showed that lovastatin inhibited proliferation of breast cancer cell lines. breast cancer MDAMB231 cells were more sensitive to its effects, and in most cases lovastatin acid showed more potency towards the manipulation of protein expression than lovastatin lactone. Bhargavi *et.al*. (5), found that partial purified lovastatin from A.terreus (KM017963) (purified by adsorption chromatography) was effect on HeLa cells through its impact on mitochondrial membrane potential, quantities of cell, DNA fracture and antioxidant properties regarding on the one hand hydroxy radical scavenging and its impact on levels of diminished glutathione. While its effect in cell cycle was through cell apoptosis and cell cycle regulation in the G0/G1 phase, and the final results give leads for the anticancer impacts of lovastatin and its potential handiness in the chemotherapy of cervical malignant growth. Janani, *et.al*. (15), found that lovastatin obtained from soil fungi is exhibited significant antitumor activity against A549 cell line (from human lung). Raghunath *et.al*.,(26), demonstrated that the lovastatin extracted from fungal manifest had a strong cytotoxic activity in in-vitro culture of examined cancer cells of human (HeLa and HepG2) via apoptotic experiment.

REFERENCES

1. Ahmed, E.F., 2015. Antimicrobial and antibiofilm activity of mango seeds extract. Iraqi Journal of Science. 56(4B): 3121-3129

2. Alenghat, F.J. and A.M. Davis, 2019. Management of blood cholesterol. JAMA. 321(8):800.

3. AL-Sa'ady, A.J.R. , M.H. Al-Bahrani, and G.M. Aziz, 2018. Characterization and immobilization of peroxidase extracted from horseradish and decolorization of some dyes. Int. J. Curr. Microbiol. App. Sci. 7(6): 328-339

4. Aydin, S. and K. Kinalioglu, 2018. Comparative antimicrobial activity of crude extracts of *Protoparmeliopsis muralis* and *Parmotrema perlatum* Lichens. GU J Sci. 31(3): 687-698

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

6. Bizukojc, M. and S.A. Ledakowicz, 2007. Macrokinetic modelling of the biosynthesis of lovastatin by *Aspergillus terreus*. J. Biotechnol. 130(4): 422-35

7. Blasi, P., S. Giovagnoli, A. Schoubben, M. Ricci, and C. Rossi, 2007. Solid lipid nanoparticles for targeted brain drug delivery. Adv. Drug Deliv. Rev. 59(6): 454-77

8. Brown, A.N., K. Smith, T.A. Samuels, J. Lu, S.O. Obare, and M.E. Scott, 2012. Nanoparticles functionalized with ampicillin destroy multiple antibiotic-resistant isolates of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* and Methicillin-Resistant *Staphylococcus aureus*. Appl. Environ. Microbiol. 78: 2768–2774

9. Candyrine, S.C.L., M.F. Mahadzir, S. Garba, M.F. Jahromi, M. Ebrahimi, Y.M. Goh, A.A. Samsudin, A.O. Sazili, W.L. Chen, S. Ganesh, R. Ronimus, S. Muetzel, and J.B.

Liang, 2018. Effects of naturally-produced lovastatin on feed digestibility, rumen fermentation, microbiota and methane emissions in goats over a 12-week treatment period. 13(7): 1-19

10. Collins, G.H, P.M. Lynes, and J.M. Grange, 1995. Microbiology Methods. 7ed., Butterwort-Heinemannt Ltd, Britain, pp:175-190

11. Graziano, T.S., M.C. Cuzzullin, G.C. Franco, H.O. Schwartz-Filho, E.D. Andrade, F.C. Groppo, and K. Cogo-Muller, 2015. Statins and antimicrobial effects: simvastatin as a potential drug against *Staphylococcus aureus* biofilm. PLOS One.10(5):1-17

12. Hennessy, E., C. Adams, F.J. Reen, and F.O. Gara, 2016. Statins as next generation anti-microbials: Is there potential for repurposing?. American Society for Microbiology. Review. pp:1-46

13. Ho, M.H., C.P. Chiang, Y.F. Liu, M.Y.P. Kuo, S.K. Lin, H.Y. Lai, and B.S. Lee, 2011. Highly efficient release of lovastatin from poly(Lactic-co-Glycolic Acid) nanoparticles enhances bone repair in rats. Wiley Periodicals, Inc. J. Orthop Res. 29:1504–1510

14. Ivask, A., A. Elbadawy, C. Kaweeteerawat, D. Boren, H. Fischer, *et.al*. 2014. Toxicity mechanisms in *Escherichia coli* vary for silver nanoparticles and differ from ionic silver. ACS Nano 8(1): 374-386

15. Janani, B., G. Saibaba, G. Archunan, K. Vidhya, J. Karunyadevi, and J. Angayarkanni, 2017. Purification and cytotoxity study of lovastatin from soil fungi. Asian J. Pharm Clin Res. 10(10): 258-262

16. Jasim, A.M., and G. M. Aziz, 2019. Degradation efficiency of phenolic compounds using immobilized peroxidase purified from soybean. Iraqi Journal of Agricultural Sciences. 50(3):928- 935

17. Klawitter, J., T. Shokati, V. Moll, U. Christians, and G. Klawitter, 2010. Effects of lovastatin on breast cancer cells: a proteometabonomic study. Breast Cancer Research. 12(16): 1-20

18. Lewington S., G. Whitlock, R. Clarke, P. Sherliker, J. Emberson, J. Halsey, N. Qizilbash, R. Peto, and R. Collins, 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 370 (9602): 1829–39

19. Maeh, R. K., A.I. Jaaffar, and K.F.AL-azawi, 2019. Production of Junipers extract and detection of its antimicrobial and antioxidant activity. Iraqi Journal of Agricultural Sciences. 201 9:50(4):1153-1161

20. Masadeh, M., N. Mhaidat, K. Alzoubi, S. Al-azzam, and Z. Alnasser, 2012. Antibacterial activity of statins: a comparative study of Atorvastatin, Simvastatin, and Rosuvastatin. Annals of Clinical Microbiology and Antimicrobials. 11:13:1-5

21. Mielcarek, J., M. Naskreni, and P. Grobelny, 2009. Photochemical properties of simvastatin and lovastatin by radiation. J. Thermal Analysis Calorimetry 96: 301-305

22. Muhammad, H. and S. Muhammad, 2005. The used of *Lawsonia inermis* Linn.(Henna) in the management of burn wound infections. Afr. J Biotechnol. 4(9):934-937

23. Obeidat, M., M. Shatnawi, M. Al-alawi, E. Al-Zubi, H. Al-Dmoor, M. Al-Qudah, J. El-Qudah, and I. Otri, 2012. Antimicrobial activity of crude extracts of some plant leaves. Research J. Microbiology.7: 59-67

24. Oshim, I.O., C.O. Desmond, R.A.U. Nwobu, U.M. Ezugwu, and E. U. Urama, 2016. Kinetics of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of *Vernonia amygdalina* (Bitter leaf) on microorganisms isolated from wound infections. International J. Surgery Research, 5(1): 8-14.

25. Peter, H. and M.D. Jones, 2003. Comparing HMG-CoA reductase inhibitors. Clin. Cardiol. 26(I): I-15–I-20

26. Raghunath, R., A. Radhakrishna, J. Angayarkanni, and M. Palaniswamy, 2012. Production and cytotoxicity studies of lovastatin from *Aspergillus niger* PN2 an endophytic fungi isolated from Taxus baccata, IJABPT. 3(3): 562– 570

27. Rashid, H. 2017. Umamaheswari G. evaluation of the cytotoxic effect of ginger extract against prostate cancer modal using *in vitro* study. W. J. Pharm.Scie. 6(12): 1044-1053

28. Rogowska, A., K.R. Nska, P. Pomastowski, J. Walczak, V. Railean-Plugaru, M.B. Forajta, and B. Buszewski, 2017. Silver nanoparticles functionalized with ampicillin. Electrophoresis. 23:1-8

29. Xiangli, Q., L. Zhe, Z. Yinglin, and Z. Zhengjia, 2010. Immobilization of activated sludge in poly (*ethylene glycol*) by UV technology and its application in micro-polluted wastewater. Biochem Eng J. 50:71–76

30. Zipp, F., S. Waiczies, O. Aktas, O. Neuhaus, B. Hemmer, B. Schraven, R. Nitsch, and H.P. Hartung, 2007. Impact of HMG-CoA reductase inhibition on brain pathology. Trends in Pharmacological Sciences. 28(7): 342-349.