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INVESTIGATION FROM PRODUCE OF LOVASTATIN COMPOUND FROM THE FUNGUS (*PENICILLIUM ITALICUM*) AND EVALUATION OF ITS EFFECTIVENESS AGAINST THE FUNGUS (*ASPERGILLUS FUMIGATUS*)

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ABSTRACT

This study was carried out to investigate a possibility of the fungus (*Penicillium italicum*) to produce lovastatin compound by using a Solid State Fermentation (SSF) and rice powder as a substrate in it, and evaluate its effect with concentrations of (0.25, 0.5, 1, 2) mg/ml in inhibiting the growth of isolation of the fungus (*Aspergillus fumigatus*). The analysis of the SSF product by using ATR-FTIR showed presence several peaks belong to functional groups contain in lovastatin compound such as (alcohol O-H, C-H, C-O-H, and C-O-C).The study showed that the efficacy of SSF product in inhibiting the growth of studied fungus varied according to concentration compared to control, where the highest rate of inhibition reached 79% at concentration 2 mg/ml.

INTRODUCTION

Statins are a group of drugs which used to reduce blood cholesterol with ratio 20-60 % by inhibiting the synthesis of 3-Hydroxy-3-Methyl Glutaryl COA reductase, which is the main enzyme in cholesterol biomimulation [21]. The chemical synthesis of statins is made of units of acetate that are bound together to form chains known Polyketide. On the other hand, fungi produce a wide range of biologically active compounds, some of them possess important biological activities such as antibiotics, and among the important fungal metabolites (statins), lovastatin which prepares vital source, while others produce from chemical modifications to it, such as pravastatin, rosuvastatin, atorvastatin, simvastatin, and fluvastatin [1-3]. Lovastatin can be obtained from different genera and species of fungi such as, *Aspergillus*, *Monascus*, *Paecilomyces*, *Trichoderma*, *Agaricus* and etc… [1-3-4-18-23].

Lovastatin can be produced from fungi by using one of fermentation methods (Sub-Merged Fermentation (SMF), Solid Substrate Fermentation (SSF), the solid method fermentation has better advantages than submerged fermentation (maximum utilization of the substrate, provides a good environment for fungi growth, and increases the production of statins) [8-10-13-16]. Example of the substrates which use in SSF to produce lovastatin include: wheat bran, rice-bran, orange peels, cereal peels [5-9-17-20].

In this study, the possibility of the fungus (*Penicillium italicum*) to produce lovastatin compound will be done by using a Solid Substrate Fermentation method (SSF) and rice powder as a substrate in it, and also the effectiveness of the SSF product will be tested in inhibiting the growth of isolation of the fungus (*Aspergillus fumigatus*).

2. Materials and methods

2.1. Microorganism

Penicillium italicum was isolated from soil, took a sample of the soil and planted on the potato dextrose agar (PDA) and incubated at 25^0C for a week, after that the studied fungi purified, then stored in the refrigerator at 4^0C in tubes contain (PDA) culture.

2.2. Solid state fermentation

In this study, rice powder was used as a substrate to produce lovastatin, was dried at 70^0C for 24 h, cooled and grounded .A quantity of 10 g of solid substrate was taken in a 250 ml of conical flask and moistened with water to maintain the moisture at 70%(v/w). Then, 40.05 g/l of potato peels powder (as a carbon source), 15 g/l of milk powder and 5.5 g/l of yeast powder (as nitrogen sources) were added to flask. Then, the culture of fermentation autoclaved at 121^0C for 20 min, cooled to room temperature. After that flasks were inoculated with 2 mL of fungal spore suspension (10^6 spore/ml). The pH was adjusted to 6.0 with 1M HCL. Then, Flasks were incubated at 25^0C for 7 days [7-13].

2.3.Extraction of lovastatin

At the end of SSF, the fermented material was dried at 60^0C for 24 h, powdered, and 4 g of the powdered material was extracted with ethyl acetate (pH 3.0) in 250 ml Erlenmeyer's flasks. It was then, incubated at 28^0C in rotary shaker for 2 h. Then the mixture was centrifuged at 1500 rpm for 20 min. Filtration was done using Whatman filter paper No.1 for separation of fungal cell biomass from the filtrate. The supernatant concentrated in a rotary vacum evaporator at 40°C. The crude extract kept in the refrigerator in sealed and sterile glass containers until use [19].

2.4. UV/Vis spectrometer analysis

The estimation of lovastatin was calculated spectrophotmetrically by Double beam UV/Vis spectrometer (Shimadzu Model LT 1700) within range (200-400)nm

2.5.ATR-FTIR analysis of fermented product

Final confirmation of lovastatin in the prepared sample was done by using FTIR/Diamond ATR, ATR was fitted with a single bounce diamond at 45˚internally reflected incident light providing a sampling area of 1 mm in diameter with a sampling depth of several microns. A small amount of the sample was directly placed on the diamond disk. Sample was scanned for absorbance over the range from 4000 to 400 wave numbers (cm^{-1}) at a resolution of $1cm^{-1}$ [14].

2.6. Antifungal activity of fermented product

The effect of fermented product was examined in inhibiting the growth of fungi (*A.fumigatus*) by the Poison Food Method [15] With some appropriate modifications. The sample was prepared at concentrations (2.5, 5, 10, 20) mg/ml. Then added 1 ml of each of the concentrations to 10 ml of the PDA culture to become the final concentrations (0.25, 0.5, 1, 2) mg/ml stirred well, then poured in plastic petri plates, left to harden at the laboratory temperature, then took a disc with a diameter of 5 mm from the sides of Colony of the studied fungus by using a sterilized needle and placed in the middle of each plate. The control plates were made by Cultivate the studied fungus on the PDA culture without adding any extract, the plates incubated at 28 ± 2^0C for 7 days [6].

The experiment was performed with three replicates for each sample and each concentration alonely and the control plates, then the colony diameter was measured in the middle of each dish, took the average growth rate of the fungal colonies of the three replicates and then calculated the inhibiting percentages according to the following equation [25]:

Percentage of inhibition = (The average diameter of the control colony – The average diameter of the treated colony) x100 / The average diameter of the control colony.

3. Results and discussion

3.1. Isolation and characterization of fungal isolate from soil sample

The fungal isolate was characterized by using standard micro biological methods such as morphological and microscopic properties (Colony colour, Conidia, PhialideConidiophores, and Metulae). Characterized and identified fungal isolate was shown in (Figure1, Table 1).

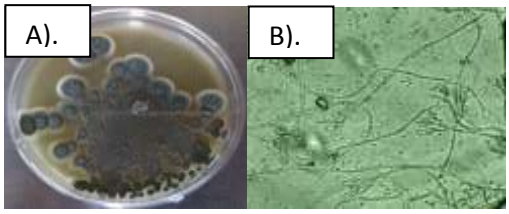

Figure1. Characterization of *Penicillium italicum.* A.*P.italicum* colonies on PDA plate showing Olive green color; B. conidiophores of *P.italicum* (40 x 10).

3.2 UV/Vis spectrometer analysis of fermented product (lovastatin)

The analysis by spectrophotometer showed an absorption peak at a wavelength 238 nm, which corresponds to the absorption peak of the reference lovastatin compound [6].

3.2 ATR-FTIR analysis of fermented product (lovastatin)

The ATR-FTIR spectra of *Penicillium italicum* ssf extract (lovastatin**)** was shown in figure2. The spectrum presented characteristic peaks at 3514.63 cm^{-1} (alcohol O-H stretching vibration), 2950 cm^{-1} (olefinic C-H stretching vibration), 2360.44 cm^{-1} (methyl and methylene C-H asymmetric stretching), 1585.2 cm^{-1} (lactone and ester carbonyl stretch), 1440.87 cm^{-1}, 1375 cm^{-1} (methyl and methylene bending vibration), 1342.55cm^{-1} (C-O-H bending vibration), 1050.88 cm^{-1} (ester C-O-C symmetric bend), and 750.33 cm^{-1} (meta disturbed benzene-strong) confirms presence of lovastatin in the SSF sample.

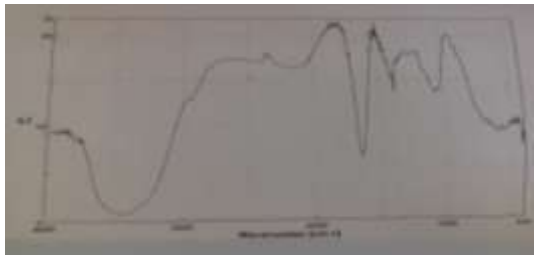

Figure 2. ATR-FTIR analysis of *Penicillium italicum* SSF extract.

3.3 The activity of fermented product (Lovastatin) against *A.fumigatus*:

Table 2. shows that, Effectiveness varied according to the concentration, where the concentration 2 mg/ml showed the highest efficacy in inhibiting the growth of *A.fumigatus* by a ratio reached 79%, the results of the study was agreed with several studies that showed the efficacy of statins; (lovastatin, atorvastatin, simvastatin, pravastatin, rosuvastatin) against some microorganisms [2-6-24], the exact mechanism of antifungal activity exhibited by the statins is unknown, The direct cytotoxic effect of statins may possibly relate to the antifungal activity by presence phenolic compounds and aromatic alcohols are reported to have antimicrobl activity mediated by growth inhibition, lethal effect, and cytologic damage [11]. The presence of phenolic hydroxyl groups in lovastatin may correlate with their antifungal activity.

Figure3. shows colonies of *A.fumigatus* were treated by different concentrations of lovastatin produced by *P.italicum* compared to control sample.

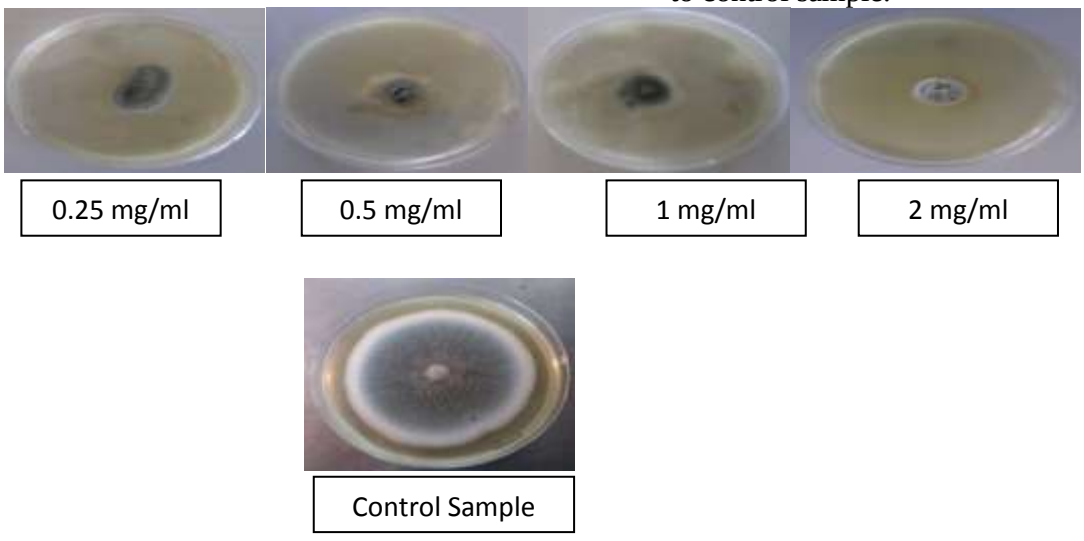

Figure 3. Colonies of *A.fumigatus* were treated by different concentrations of fermented product compared to control sample

Table 1. Morphological and microscopic properties of fungal isolate

Fungal isolate	*P.italicum*
Colour of top side of colony	Greenish grey
Conidia	Smooth, ellipsoidal,(3.5-4)µ
Phialide	Cylindrical,(8-10)µ
Conidiophore	Terverticillate
Metulae	Cylindrical,(12-14)µ

Table 2. The activity of fermented product against *A.fumigatus*

Control	**0.25 mg/ml**	**0.5 mg/ml**	**1 mg/ml**	**2 mg/ml**
DIZ %	DIZ %	DIZ %	DIZ %	DIZ %
8.25 0	3.25 61	2.31 72	2.15 74	1.74 79

DIZ= Diameter of inhibition zone; %= inhibiting ratio.

Conclusion:

This study showed presence lovastatin compound as fermented product by *Penicillium italicum* which has antifungal properties. so that, we suggest to do further studies to screen the production of lovastatin by other fungi and test its effectiveness in inhibiting microorganism invitro and invivo.

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