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Research Article

IN VITRO ANTICANCER POTENTIAL OF STATIN FROM *ASPERGILLUS TAMARII* GRD119

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ABSTRACT

Objectives: Fungi have been largely targeted for their biopotential mainly in the pharmaceutical industry. Recently, there has been an increase in screening of biopharmaceuticals from microorganisms so as to eliminate side-effects and chemical damages from non-natural sources.

Methods: In the present study, *Aspergillus tamarii* GRD119 (JX110981) was screened for the production of statin by conducting bioassays against *Candida* and filamentous fungi.

Results: Based on the results obtained showing effective inhibition potential against *Candida* spp. and *Aspergillus fumigatus*, further analysis of purification of statin by several chromatographic analyzes such as column chromatography, thin layer chromatography (TLC), high-performance TLC and high-performance liquid chromatography was conducted. The extracted statin shows strong cytotoxic activity against HEP G2 cell line.

Conclusions: These results designate the fungal strain and the statin produced can serve as candidates for potential genetic transformation and further structural analysis respectively.

Keywords: *Aspergillus tamarii*, chromatography, purification, statin, cytotoxicity.

INTRODUCTION

Statins are compounds of natural origin that are biosynthesized as secondary metabolites of several filamentous fungi. The pharmaceutical component of statin acts as an inhibitor for hydroxyl 3-metylglutaryl – Coenzyme A reductase in the biosynthesis of cholesterol [1]. After successful clinical trials of the natural statins, pharmaceutical companies introduced more effective and safer, fully-synthetic statins [2]. All natural statins possess a common polyketide portion, a hexahydro naphthalene ring system to which are attached different side chains. Now-a-days lovastatin and its semisynthetic derivatives are very important drugs since the mortality of heart disease is becoming relatively high.

The increasing toxic side effects of antifungal drugs have augmented the focus on safely applicable drugs with the potent antifungal property. Statins, originally used as cholesterollowering agents, have now been researched for their potential antifungal property alone or in combination with other antifungal agents. Microbiologically active against yeast and filamentous fungi, ineffective combinatorial concentrations statins have found to treat invasive fungal infections; thus confirming its synergistic combination as a new applicable therapy [3]. Also, statin has been used in the biomedical applications such as treating coronary heart diseases, renal diseases, Alzheimer's disease, and bone fractions [4].

The production of effective and safe pharmaceuticals requires purity of drugs as an important factor that is either by chromatography or solvent based extraction procedure. Due to their ease in purification solvent extraction although is used regularly, chromatographic techniques have been favored with regard to purity. Chromatogrphic purification is achieved either by high-performance liquid chromatography (HPLC), displacement chromatography, preparative thin layer chromatography (TLC), or elution chromatography [5].

In the present study, production of statin by *Aspergillus tamarii* GRD119 (JX110981) was initially screened by a simple extraction procedure and the activity of the statin was analyzed using a bioassay using *Candida albicans* (MTCC 183) and *Aspergillus fumigatus* (JX041523). Confirmation of the compound obtained as statin was done using TLC, HPLC and high-performance-TLC **(**HPTLC) techniques followed by an attempt to screen for the anticancer property.

MATERIALS AND METHODS

Maintenance of microbial isolates

A. tamarii GRD119 (JX110981) and *A. fumigatus* (JX041523) was subcultured on potato dextrose agar (PDA) (Himedia, India) incubated for 4 days at 35°C and stored at 4°C for further study.

C. albicans (MTCC 183) was obtained from IMTECH, Chandigarh, India. The organism was initially incubated for 48 hrs at room temperature in nutrient broth (Himedia, India) for reviving the lyophilized culture. Further sub culturing was done on YEPD agar (yeast extract - 3 g, peptone - 10 g, dextrose - 20 g, agar - 15 g, distilling water - 1000 ml, pH - 6.5) for 48 hrs and YEPD broth (yeast extract - 3 g, peptone - 10 g, dextrose - 20 g, distilled water - 1000 ml, pH - 6.5) for 24 hrs at 37°C and stored at 4°C.

Screening for aflatoxigenic potential of *A. tamarii* GRD119 (JX110981)

The strain was inoculated at the center of the solidified PDA medium and incubated at 25°C. After 3 days of growth, dishes were placed upside down and a drop (0.2 ml) of 25% ammonia solution was put into the lid of the petri dish to observe if any change in color was observed on the reverse side of the colony [6].

Screening of *A. tamarii* GRD119 fungal isolates for statin production

The maintained *A. tamarii* GRD119 cultures were subcultured in petri plates containing PDA medium and incubated for 7 days. The colonies obtained were scraped and transferred to a sterile falcon tube. 1 ml of ethyl acetate was added for extraction of statin and incubated at 60°C for 10 minutes followed by vortexing for 5 minutes. This suspension was further heated at 50°C for 5 minutes and vortexted for 2 minutes. The extract obtained was centrifuged at 10,000 rpm, 40°C for 15 minutes.

The aqueous layer obtained after centrifugation was screened for the presence of statin.

Bioassay for confirming the activity of statin by growth inhibition of *C. albicans* and *A. fumigatus*

A rapid method to identify and demonstrate the production of statin by using microorganisms is favored as it involves less expense and less labor. Using *C. albicans* (MTCC 183) and *A. fumigatus* (JX041523): Strains were grown on potato dextrose broth (Himedia, India) containing various concentrations of 50, 100, and 250 mg/ml of cholesterol and incubated at 35°C for 48 hrs and 5 days, respectively. 25 ml of YEPD agar was prepared to which 100 µl of aqueous extract from *A. tamarii* GRD119 was added onto which the cultures from cholesterol containing plates were streaked [7].

Minimum inhibitory concentration (MIC)

Direct method

2 ml of potato dextrose broth (Himedia. India) was taken in each test tube, and 100 µl of an overnight culture of *C. albicans* was added. 5-100 µg of the fungal statin were added to each tube at a gradient of 10 µg. A tube was maintained as a control without adding the fungal extract. All the tubes were incubated for 24 hrs at 37°C. The turbidity was measured spectrophotometrically at 600 nm.

Serial dilution method

A test tube was maintained with 2 ml YEPD medium (HiMedia, India), 100 µl *C. albicans* culture and 100 µg of fungal statin. The tubes were serially diluted seven times with final concentrations of 100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg, 3 µg, and 1.5 µg. A positive control (2 ml YEPD medium + 100 µl *C. albicans* culture) and negative control (2 ml YEPD medium + 100 µl fungal statin) were maintained. All the tubes were incubated at 37°C for 24 hrs, and the turbidity was measured at 600 nm spectrophotometrically.

Bioassay for confirming the concentration of statin using various volumes of fungal extract against *C. albicans*

Using *C. albicans* (MTCC183): Wells were punched in solidified YEPD agar plates swabbed with *C. albicans* (MTCC183) using a sterile borer of 8mm diameter. 50 µl, 75 µl, 100 µl, and 125 µl of the aqueous extract obtained was added to the wells and incubated at 35°C for 24 hrs. Ethyl acetate was used as control [7]. A standard curve was prepared, and the volumes of each extract were compared for determining the concentration of statin present.

Extraction of statin by fermentation

Spores of 5 days old culture of *A. tamarii* GRD119 (JX110981) were inoculated into the fermentation broth (dextrose - 10 g, peptone - 10 g, KNO_3 - 2 g, $NH_4H_2PO_4$ - 2 g, $MgSO_4.7H_2O$ - 0.5 g, $CaCl_2$ - 0.1 g) and incubated for 7 days in a rotary shaker at 180 rpm for 7 days. The broth was acidified to pH 3.0 with 10% HCl. The acidified broth was extracted with equal volume of ethyl acetate under shaking condition for 2 hrs. The samples were subsequently centrifuged at 1500 rpm for 15 minutes, and the organic phase was collected for further steps of purification.

Purification of statin

Purification of statin by column chromatography

Silica gel (60-120 mesh size) was continuously stirred with the slurry of crude extract in a porcelain dish until it was adsorbed on silica gel. The completely dried slurry was loaded on the top of the column (60 cm × 3.5 cm) pre-packed with silica gel of 60-120 mesh size (stationary phase) with benzene. Polarity based elution was carried out using 100% benzene followed with acetonitrile in benzene up to 100% acetonitrile by 5% increment. The polarity was further increased with water in acetonitrile up to 100% water similar to the previous step. Each chromatographic fraction (500 µl) collected with different polarities was concentrated (25 µl) and then lactonized with 1% trifluoroacetic acid and each fraction obtained using various ratios of both solvents were collected [8]. Each fraction was further analyzed against *C. albicans* (MTCC 183) for checking the maximum effective fraction of statin extracted and compared with a commercial statin.

Preparation of sample for TLC, HPLC and HPTLC

The sample obtained after purification by column chromatography showing effectiveness against *C. albicans* (MTCC 183) was subjected to lyophilization for concentrating the sample obtained. 500 µl of the sample was taken in an Eppendorf tube (1.5 ml) and placed at −80°C overnight and subjected to lyophilization in Christ LCG Lyochamber Guard 121550PMMA (Germany). The powdered sample of the statin obtained was subjected to further analysis.

Analysis by TLC

Thin layer chromatographic plates (20 cm × 20 cm glass plates) were coated with silica gel-G (200 mesh) to 1 mm thickness using a spreader. After air drying, the plates were activated by exposure at 120°C for 2 hrs in a hot air oven. 10 mg of powdered fungal statin sample obtained after lyophilization and the standard statin (Dr. Reddy's Laboratories, India) was dissolved in 1 ml of ethyl acetate and maintained as a stock solution. A quantity of 20 µl from the stock of the crude extract was spotted. The plates were developed in a solvent system of dichloromethane: ethyl acetate (70:30 v/v). Individual spots on the TLC plates were marked and Rf values calculated after the plates were stained with iodine vapor and observed under UV light. Each spot was collected from the plates and stored in a clean glass vial. Standard statin was also spotted for Rf comparison. The plate was developed in the mobile phase for at least three times [9], and the Rf value was calculated by the following equation:

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

HPTLC

The spot obtained after TLC analysis of the fungal extract was scrapped and dissolved in 1 ml of ethyl acetate. The mixture was centrifuged at 3000 rpm for 5 minutes, and the supernatant was used as test solution for HPTLC analysis. 3 µl of fungal statin solution and 3 µl of standard solution were loaded as 5 mm band length in the 3 × 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Chloroform: Methanol – 9.5:0.5) and the plate was developed up to 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV 366 nm. The developed plate was sprayed with respective spray reagent (Iodine vapours) and dried at 100°C in a Hot air oven. The plate was photo-documented in a visible light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Statin quantification and analysis

Pharmaceutical-grade commercial statin (lactone form) was used to prepare the standards for HPLC analyzes. Prior to use, the lactone form was converted into its β-hydroxyacid form by dissolving the commercial statin in a mixture of 0.1 mol/L sodium hydroxide and ethanol (1:1, v/v) followed by heating the solution at 50°C for 20 minutes and neutralizing it with one drop of 1N hydrochloric acid. HPLC was performed using Beckman Ultrasphere ODS (250 × 4.6 mm I.D., 5 µm) column mounted in a Shimadzu model LC10 liquid chromatograph equipped with a Shimadzu MX-10Av diode array detector (Shimadzu Corp., Kyoto, Japan). The mobile phase was a mixture of acetonitrile and 0.1% phosphoric acid (60:40, v/v) with a flow rate of 1.5 ml/minutes and detection wavelength at 238 nm with the sample injection volume was 20 µl. After derivatization, the plate was fixed in scanner stage (Camag TLC scanner 3) and scanning was done at UV 500 nm. The peak

table, peak display and peak densitogram were noted. The software used was won CATS 1.3.4 version.

Cell cytotoxicity analysis

HEP G2 cell line was passaged thrice and the cells were split into 1:2, 1:3 ratio for cytotoxicity studies by a plating method. One ml of each suspension was pour into 24-well plates and kept in desiccators in 5% CO_2 atmosphere. Statin was added into wells seeded at 10^6 cells/well and cell death, and cell viability was estimated by MTT assay. 200 µl of MTT at 5 mg/ml concentration was added followed by 1 ml of dimethyl sulfoxide and left for 45 seconds for purple color formazon formation. This color was read at 595 nm and cell viability was calculated as

Cell viability (%) = Mean OD/Control OD × 100

RESULTS

Screening for the non-aflatoxigenic potential of the strain was carried out as aflatoxin producing strains of *A. tamarii* GRD119 are not suitable for secondary metabolite productions. Several detailed analysis of the aflatoxin producing strains is to be carried out inorder to make the secondary metabolites suitable for application purposes without the presence of aflatoxin or toxin mycotoxins. On exposure to ammonia vapor, no change in color was observed, thus confirming the non-production of aflatoxins by *A. tamarii* GRD119 (JX110981).

Strains were initially grown on various concentrations of cholesterol with PDA, and the spores were transferred to a plate with statin incorporated solidified PDA. The cultures that were exposed to the higher concentrations of cholesterol were shown higher growth in the presence of statin. The strain of the highest concentration of cholesterol was found to shield the effect of statin and promote growth due to the ineffectiveness of the statin against high concentrations of cholesterol present (Figs. 1 and 2). The ethyl acetate broth from *A. tamarii* GRD119 have found to cause strong inhibition of growth in *Candida* species and *A. fumigatus* (JX041523) strains. The results indicate that statins may have a potential role as antifungal agents (Table 1).

The MIC represents the concentration of antimicrobial at which there is complete inhibition of growth. In reading the end points, a barely visible haze of growth is disregarded. MICs comparison of both methodologies demonstrated 100% categorical agreement of the results obtained for both the direct and serial dilution method. 50µg of the fungal extract showed the MIC and was used as the minimum concentration of the extract used for bioassays (Table 2).

Table 1: Bioassay using *C. albicans* MTCC183 showing diameter of inhibition zone using fungal extract on comparison with commercial statin

Serial number	Concentration of commercial statin (µg/ml)	Diameter of inhibition zone using commercial statin (cm)	Concentration of fungal statin (µg)	Diameter of inhibition zone using extracted isolate (cm)
1	50	1.8	50	1.2
2	75	2.1	75	1.6
3	100	2.6	100	1.9
4	125	3.2	125	2.8

C. albicans: Candida albicans

Table 2: MIC of fungal statin against *Candida albicans* MTCC183

Direct method		Serial dilution method	
Concentration of fungal statin (µg)	Optical density at 600 nm	Concentration of fungal extract (µg)	Optical density at 600 nm
5	0.992	1.5	1.101
10	1.082	3	1.128
20	1.058	6.25	1.046
30	0.891	12.5	0.932
40	0.911	25	1.046
50	0.558	50	0.205
60	0.459	100	0.144
70	0.129	-	-
80	0.109	-	-

MIC: Minimum inhibitory concentration

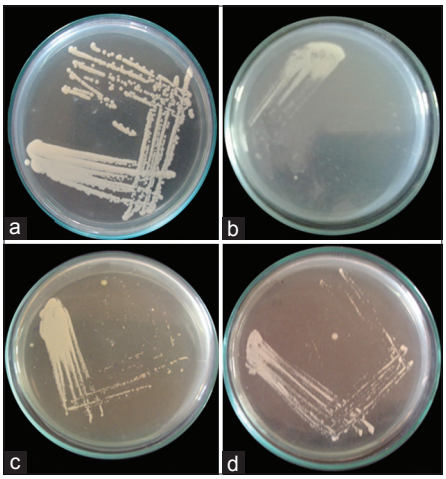

Fig. 1: Bioassay for confirmation of statin potential in the fungal extract from *Aspergillus tamarii* GRD119 against *Candida albicans* MTCC183. (a) Growth of *C. albicans* in presence of 250 mg/ml of sterol and no stain; (b) Growth of *C. albicans* in presence of 50 mg/ml of sterol and 100 µl of fungal statin; (c) Growth of *C. albicans* in presence of 100 mg/ml of sterol and 100 µl of fungal statin, (d) Growth of *C. albicans* in presence of 250 mg/ml of sterol and 100 µl of fungal statin; (a and d) Fig. 1d shows decrease in growth of *C. albicans* in the presence of statin but same amount of sterol as in Fig. 1a

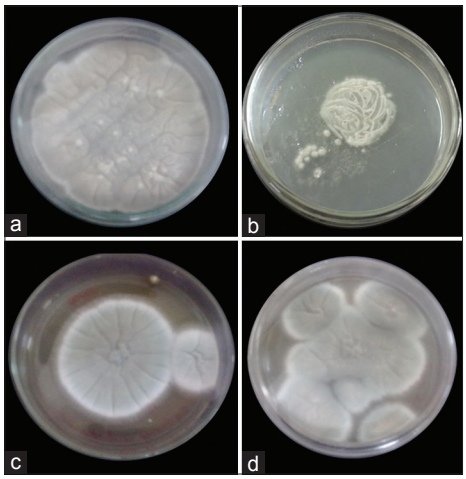

Fig. 2: Confirmation of statin potential in the fungal extract from *Aspergillus tamarii* GRD119 against *Aspergillus fumigatus* (JX041523). (a) Growth of *A. fumigatus* in presence of 250 mg/ml of sterol and no stain; (b) Growth of *A. fumigatus* in presence of 50 mg/ml of sterol and 100 µl of fungal statin; (c) Growth of *A. fumigatus* in presence of 100 mg/ml of sterol and 100 µl of fungal statin; (d) Growth of *A. fumigatus* in presence of 250 mg/ml of sterol and 100 µl of fungal statin; (a and d) Fig. 1d shows decrease in growth of *A. fumigatus* in the presence of statin but same amount of sterol as in Fig. 1a

Purification of statin by column chromatography yielded high concentrations of statin effective against *C. albicans* (MTCC 183). The column first eluted with benzene and gradient 5% of acetonitrile increase was used for elution. Following this, the addition of gradient 5% mobile phase with water was done until 100% polarity of acetonitrile was reached. Each of the fractions obtained on elution was further tested against *C. albicans* to find the most effective fraction of statin obtained. The highest zone of inhibition of 2 cm was obtained when the elution was carried out using 100% polarity of acetonitrile. This purified fraction obtained was used for further chromatographic analyses.

The presence of statin from acidified culture media could not be detected by the TLC system mentioned in this study, however when treated with an organic solvent system of ethyl acetate statin could be visualized. This is due to the presence of statin in open hydroxyl form which in the presence of ethyl acetate changed into lactone form that was detected by the TLC system (Fig. 3). The use of ethyl acetate could be attributed to the good solubility of statin and concentrating potential of the organic solvent that increased the sensitivity of this detection methodology.

HPTLC profile of commercial and extracted statin was recorded. Blue zone was detected in UV (238 nm) and under visible light after exposure to iodine vapors (after derivatization) in the chromatogram confirming the presence of statin. The extracts were run along with the standard statin obtained commercially. The extract from fungus shows the presence of polyphenols in the chromatograph in UV as well as after derivatization. The peak height of the respective polyphenols was also given in the Fig. 4a and b confirming that the fungal extract from *A. tamarii* GRD119 contains two types of statin.

The purpose HPLC analysis of any drugs is to confirm the identity of a drug and provide quantitative results. In the present study, the statin peak was detected at a retention time (RT) of 7.064 minutes. The effective fraction obtained from the chromatographic column was analyzed for the presence of statin. The RT of the sample (RT=7.015) resembled with standard lovastatin (RT=7.064 minutes) (Fig. 5a and b) with the mean RT for statin (acid form) was 7 minutes. The presence of statin, therefore, is confirmed although the type of statin needs further validation.

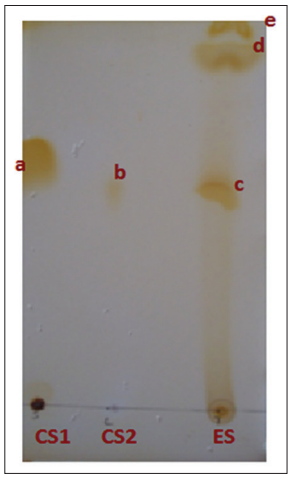

Fig. 3: Determination of statin from *Aspergillus tamarii* GRD119 in thin layer chromatography. CS1 - 25 µg/ml commercial standard simvastatin (0.625 cm); CS2 - 10 µg/ml commercial standard lovastatin (0.59 cm); ES - Fungal statin (0.59 cm); a, b, c, d - Distance travelled by solute; e - Distance travelled by solvent

HEP G2 cell cytotoxicity determined by MTT assay revealed a moderate reduction of viable cells by fungal statin and standard statin used in the study. In order to clarify the results of the effect of statin, the cells were seeded at high, low and IC_{50} concentration of statin that revealed dramatic effect on cellular morphology and became smaller with the reduction in the number of viable cells in a dose-dependent manner (Fig. 6).

DISCUSSION

The fungal aspergili have produced various secondary metabolites as a reaction to its biotic and abiotic environment. Statin production has been a feature of genus *Aspergillus* that has been targeted especially after data from more recent trials involving atorvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin, have since expanded the patient population shown to benefit from statin therapy [10].

The bioexperimental systems of yeasts and *Candida* strains have been used as tools to describe the potential application in clinical studies [11,12]. This emergence of biosystems to test antifungal as a mode of treatment has reinforced to using of microbiologically alternatives for effective management. The diameter of inhibition in Table 1 and Fig. 1 shows the potential presence of statin compounds using the fungal extract obtained. As cases of fungal invasions also include filamentous fungi, a study to propose the potential clinical application was undertaken using *A. fumigatus* on solidified minimal media. In the absence of statins, *A. fumigatus* exhibited robust growth with production of conidia after 4 days at 31°C. In the presence of statins, there was growth inhibition in regular *A. fumigatus* strains. This suggests to use of statin in human population could alter the normal pattern of fungal colonization clinically. Although some studies found no antifungal activity for statins [13], our study is in accordance with Macreadie *et al.* [7]. The possible negative results reported by Chin *et al.* [13] could be due to the lack of activation of the prodrug by hydrolysis.

Essential features that favor the use of TLC system in the analysis of potential pharmaceutical preparations is due to its simplicity. The RT of our study has also shown variations from the literature mentioned in Table 3 although the standard used showed similar Rf values with the fungal extract. These variations may be due to the various solvents used in all the studies mentioned.

In the last few decades, HPTLC has become known as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of marker compound and mobile phase with very less time [18]. Standard commercial statin showed a single peak in HPTLC chromatogram. After development, the plate was scanned using visible light after exposure to iodine vapor.

HPLC methods for the determination of stimulant related compounds are versatile and convenient and, therefore, applicable in the analysis of various drugs. Recruiting the presented HPLC protocol, we were able to determine lovastatin after extraction from the acidified broth mainly in lactone form. From the mentioned references in Table 4, it is seen that the statin RT of statin and sample have varied; although the statin used as standard and sample have been of the similar RT.

Three major parameters are responsible for a successful chromatographic separation or for a complete purification of an unstable protein: (i) the total time required for the respective separation step and for the whole purification procedure, (ii) the nature and intensity of interaction of the protein with chromatographic matrices, columns, tubing other proteins such as proteases etc., and (iii) the resolution of the chosen procedure. The chromatographic methods used were validated and found to be simple, rapid, precise,

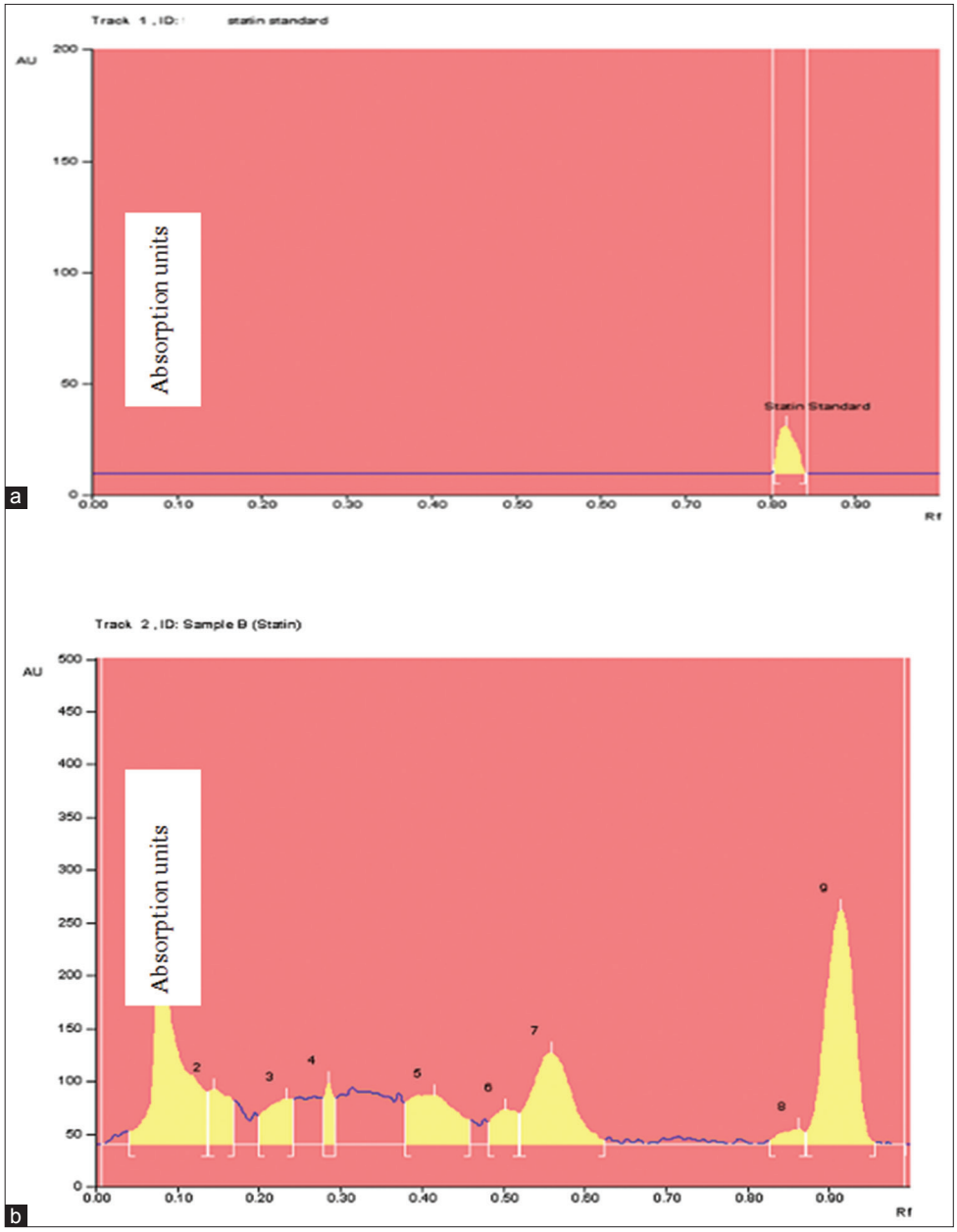

Fig. 4: (a) High-performance thin layer chromatography (HPTLC) profile for commercial standard statin. Rf values for the commercial standard statin. (b) HPTLC profile for fungal statin. Rf values for the commercial standard statin

Table 3: Literature data regarding the Rf values of statin from fungal sources by TLC

Standard used	Solvent system	Rf value of standard	Sample	Rf of fungal statin	References
Lovastatin	Dichloromethane: Ethyl acetate (70:30)	0.5	Fungus	0.5	[14]
	Toluene: Ethanol (80:20)	0.701	Fungus	0.70	
	Ethyl acetate: Hexane: Acetic acid (70:30:6)	0.56	Fungus	0.56	
	Dichloromethane: Acetic acid (85:50)	0.6	Fungus	0.59	
Lovastatin	Bezene: Acetone: Acetic acid (70:30:3)	0.66	Fungus	0.66	[15]
Lovastatin	Dchloromethane: Ethyl acetate (70:30)	0.24	Fungus	0.25	[16]
Lovastatin	Dichloromethane: Ethyl acetate (70:30)	0.67	Fungus	0.67	[17]
	Ethyl acetate: Hexane: Acetic acid (70:30:6)	0.55	Fungus	0.55	
	Acetonitrile: Hexane: Acetic acid (30:70:6)	0.50	Fungus	0.50	

TLC: Thin layer chromatography

more accurate, reliable, least time-consuming TLC, HPTLC, and HPLC methods. These methods give good resolution for statins with a short analysis time (<9 minutes).

Although statin have been extensively research on its property of treating hypercholesterol, our results have indicated anticancer property as cell proliferation was inhibited, and changes in cell

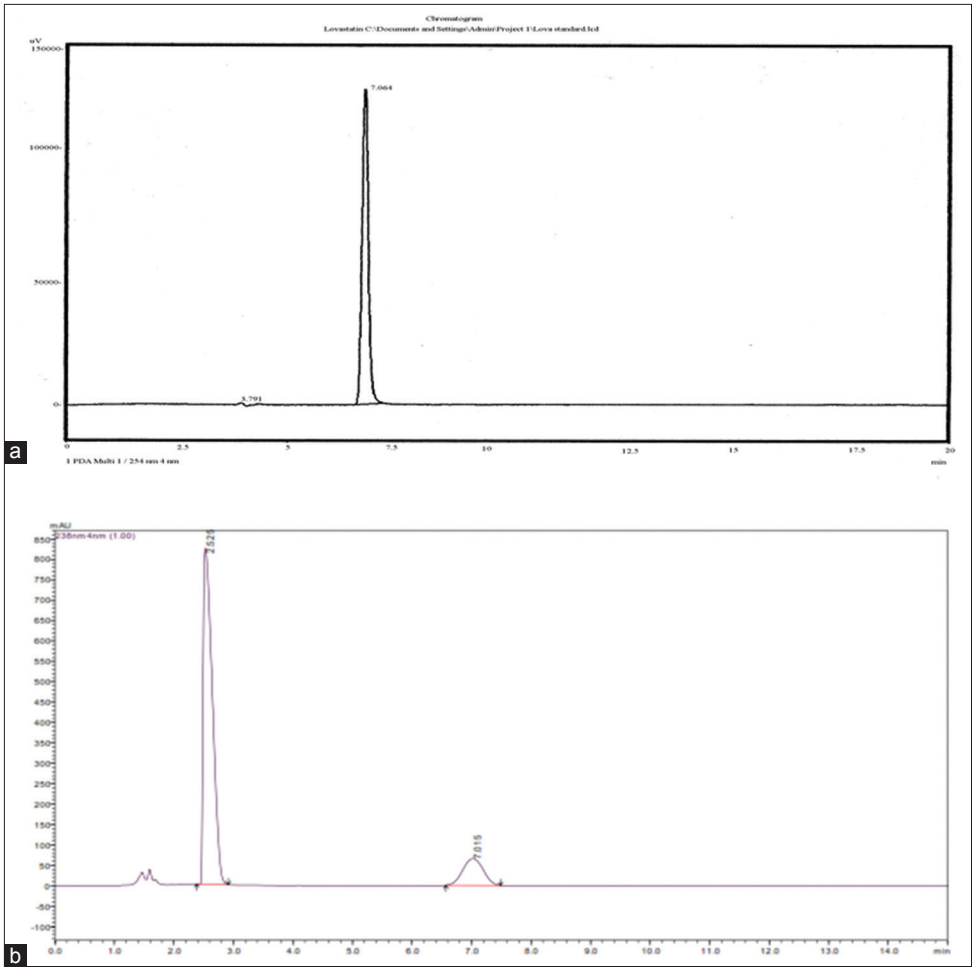

Fig. 5: (a) High-performance liquid chromatography (HPLC) showing the concentration of commercial statin. (b) HPLC Chromatogram showing concentration of crude fungal statin

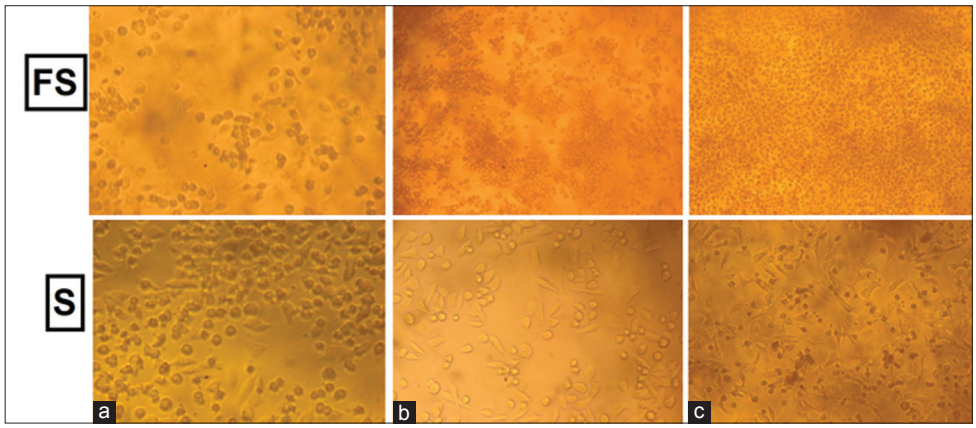

Fig. 6: Dose-dependent cytotoxicity of fungal statin by MTT assay. FS: Fungal statin, S: Standard; (a) High concentration of statin, (b) IC_{50} value of statin; (c) Low concentration of statin

morphology were observed. Statins in higher concentrations revealed higher chemotherapeutic activity. Earlier reports have suggested apoptosis of cell lines by the induction of caspases [27]. Protocols have also inferred cytotoxicity after statins were incubated for 24 hrs with HeLa cells [28]. Our results are also in accordance with myeloma researchers who had used chemotherapeutic agents in combination with statins [29] and other dose-dependent studies [30].

CONCLUSION

This is the first study to report the production of statin by *A. tamarii* GRD119. This documentation of isolation, purification and anticancer property of extracted statin confirmed to be identical to that of standard statin used in this study. This fungus can further be subjected to biotransformation and genetic engineering studies that target enhanced production of statin making it a biotechnologically potential species.

Table 4: Literature data regarding the Rf values of statin from fungal sources by HPLC

Standard used	Retention time	Sample	Retention time	References
Lovastatin	1.7, 3.6, 4.27	Fungus	1.7, 3.6, 4.27	[19]
Lovastatin	7.3	Fungus	7.4, 7.5, 7.5	[5]
Lovastatin	Lactone form 5.3, Hydroxy acid 1.3	Fungus	Lactone Form 5.1	[14]
Lovastatin	13.2	Fungus	-	[16]
Lovastatin	Hydroxy acid 6.6 Lactone form 10	Fungus	Hydroxy acid 6.6 Lactone form 10	[20]
Lovastatin	Hydroxy acid 5 Lactone 10	Fungus	Hydroxy acid 5 Lactone 10	[10]
Lovastatin	Lactone form 19.1	Fungus	Lactone 19.1	[21]
Simvastatin	6.47	Medical drug	-	[22]
Simvastatin	3.106	medical drug	-	[23]
Lovastatin	7.5	Medical drug	-	[24]
Simvastatin	9.8	Medical drug	-	[25]
Simvatatin	11.8589	Medical drug	-	[26]

HPLC: High-performance liquid chromatography

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