

Elucidation of Antifungal, Antioxidant and Anticholesterol Activity of Efficiency of Fungal Statin Isolated from *Aspergillus tamarii*

Nidhiya Kizhakkootayil, Anil Kumar, Arya Radha Krishnan Krishna, Jayaprabha Chockalingam, Lavanya Bala krishnan, Navitha Thanu Janarthanam, Praveen Rama chandran, Sharba Deepa Palanisami, Sharmila Srimathi Dhanaraj, Sindhya Joseph, Swathy Krishna Jayalekshmi, Trisha Mary Pandipilly Antony and Suganthi Ramasamy*
G. R. Damodaran College of Science, School of Biotechnology, 641 014, Coimbatore, India

ABSTRACT

Statins are drugs that lower the level of low-density lipoprotein (LDL) cholesterol levels as statins involve as potent inhibitors of cholesterol synthesis in blood. The use of statins in the medical field has enormous applications. Among few recent applications, Statins used in hospitals are proved to lower the risk of mortality among individuals effected with corona virus, its effective in protection against the neurodegenerative disorder, local application of statin have proved to repair bone. The present study was delineated to isolate the fungal strains and to screen statin production analyzed by growth inhibition of *Candida albicans* and *Aspergillus fumigatus*, further applied to evaluate the antioxidant and anticholesterol activity of fungal statin isolated from *Aspergillus tamarii*. This report depicts the antifungal activity of isolated fungal statin was done by well diffusion method which showed susceptibility of *Candida sp.* against the fungal statin, antioxidant potential of fungal statin was observed by DPPH (2,2 - diphenyl- 1- picrylhydrazyl) radical scavenging assay that proved to have increasing inhibition percentage with increasing concentration from 25µg/mL to 100µg/mL. Further, the anti-cholesterol analysis was done using male albino rats having high cholesterol diet proved that the high fat diet had adverse effect on the liver, while total bilirubin showed only marginal increase when compared to normal rats. Therefore, this study depicts an unprecedented work of fungal statin from *Aspergillus tamarii*, showing good potential to be used as antifungal, antioxidant and cholesterol lowering agent. Therefore, these natural statins could be used as a replacement for chemical drugs with no adverse side effects.

KEY WORDS: ASPERGILLUS TAMARII, ANTIFUNGAL, ANTIOXIDANT, ANTI-CHOLESTEROL.

ARTICLE INFORMATION

*Corresponding Author: sugantham2000@gmail.com
Received 20th July 2020 Accepted after revision 28th Sep 2020
Print ISSN: 0974-6455 Online ISSN: 2321-4007 CODEN: BBRCBA

Thomson Reuters ISI Web of Science Clarivate Analytics USA and Crossref Indexed Journal



NAAS Journal Score 2020 (4.31) SJIF: 2020 (7.728)
A Society of Science and Nature Publication,
Bhopal India 2020. All rights reserved
Online Contents Available at: <http://www.bbrc.in/>
DOI: <http://dx.doi.org/10.21786/bbrc/13.3/89>

INTRODUCTION

Secondary metabolite production, a hallmark of filamentous fungi, is an inflating area of research for the *Aspergilli*. A variety of biologically active compounds are produced by fungi, mainly by the polyketide biosynthetic pathway. Fungal polyketides comprise a very large and structurally diverse group and many display important biological properties such as antibiotic activity and other related pharmacological properties (Bedford et al., 1995). Among the fungal metabolites, statins (anticholesterol compounds) are considered as the most important class of secondary metabolites produced by the polyketide pathway. Statins shows anti-fungal effects (Tavakkoli et al., 2020).

Statins are a class of molecules with a polyketide structure, obtainable by secondary fungal metabolism, which can inhibit HMG- CoA (hydroxyl methyl glutaryl - coenzyme A) reductase activity. Thus the mechanism involved in the control of endogenous cholesterol levels by Statins ; makes the molecules suitable for therapeutic use (Alberts et al., 1980; Endo, 1985a; Alberts, 1988; Farnier and Davignon, 1998; Stein et al., 1998; Furberg, 1999; Maron et al., 2000; Chong et al., 2001; Tobert, 2003). Initially statins were discovered in fungi and for many years fungi were the sole source for the statins (Subhan et al., 2016).

Lovastatin is a naturally occurring statin obtained from different genera and species of filamentous fungi. Fungal strains including *Aspergillus*, *Penicillium*, *Monascus*, *Paecilomyces*, *Trichoderma*, *Scopulariopsis*, *Doratomyces*, *Phoma*, *Phythium*, *Gymnoascus*, *Hypomyces* and *Pleurotus* have been reported as lovastatin producers. Of many statin molecules, lovastatin and mevastatin are produced by the fungal species, while other statins like rosuvastatin, simvastatin, pravastatin, fluvastatin, atrovastin, cerivastatin are produced semi- synthetically from lovastatin (Tobert, 2003, Chakravarti and Sahai, 2004). *Pleurotus sp.* and its related strains produce higher lovastatin (Srinu et al., 2010). Recent research has focussed on the metabolic regulation of MK/lovastatin synthesis and its evidence shows that the combination of extracellular and intracellular factors is an important significance essential for MK/lovastatin metabolism (Zhang et al., 2020).

Research with fungal metabolites identified a series of compounds with potent inhibiting properties for this target enzyme HMG- CoA reductase, from which lovastatin was selected for clinical development. Cholesterol synthesis reduction by lovastatin was confirmed in cell culture, animal studies and in humans. The subsequent reduction in circulating total and low-density lipoprotein (LDL) cholesterol has also been demonstrated in animals and humans. Major mechanism of LDL clearance from the circulation were caused by hepatic LDL receptors, which proves to be the major mechanism, further research in animals has confirmed that these declines in cholesterol are accompanied by an increase in hepatic LDL receptor activity. Statin

effectively diminishes endogeneous cholesterol synthesis providing useful therapeutic properties for patients with hypercholesterolemia (Morris et al., 1993; Jenkins et al., 2005). Another interesting property of statins is that they have an effective antifungal potential against both yeast and filamentous fungi; furthermore they can be combined with clinically used antifungal agents (Galgoczy et al., 2011).

Several *in vitro* and *in vivo* studies have been carried out targeting the antioxidant potential of various types of natural and synthetic statins such as atorvastatin, (Wassman et al., 2002), pravastatin (Alanazi, 2010), fluvastatin and simvastatin (Franzoni et al., 2003). Fungi have been identified as the new sources of antioxidants due to wide production of secondary metabolites (Arora and Chandra, 2010). Recent studies reported that the preparation and characterization of nano statins using oyster mushroom (*Pleurotussajorcaju*), reduces toxicity and enhance efficacy for treatment of cardiovascular disease (Mehra et al., 2020).

MATERIAL AND METHODS

Isolation of fungal strains: The fungal isolates to be used in the study were collected from different environment sources which included sources like coffee powder, corn, coconut and cotton seeds. Coconut and corn previously infected with fungus were taken. All the samples which were incubated were further inoculated onto potato dextrose agar and incubated at room temperature until growth was observed. Cultures were subcultured to obtain pure cultures.

Screening of statin production analyzed by growth inhibition of *Candida albicans* and *Aspergillus fumigatus*: *Candida albicans* and *Aspergillus fumigatus* strains were grown on potato dextrose broth containing various concentrations of 50mg/mL, 100mg/mL and 250mg/mL of cholesterol and incubated at 35°C for 48 hours for 5 days. After 5 days of growth, the cultures were then inoculated with YEPD agar to which 100µl of aqueous extract of fungal statin from *Aspergillus tamarii* was added.

Application of the isolated fungal statin: Antifungal activity of isolated fungal statin: The anti-fungal activity of the isolated statin was compared with commercially available statins which include simvastatin, rosuvastatin, atorvastatin and lovastatin. The cultures include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium*, *Rhizopus*, *Excerothilumsp.*, *Candidaalbicans*, *Candida albicans* clinical strains, *Candida krusei*, *Candidaglabrata*, *Candida tropicalis*. The fungal cultures were maintained on potato dextrose agar at 28°C.

Anti-oxidant analysis of isolated fungal statin: The purified fungal statin and commercial statin were further evaluated for their antioxidant potential. Commercial statin and the isolated compound was dissolved in ethyl acetate and stored at 4°C. 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml of the purified statin were subjected to standard

antioxidant assays such as DPPH radical scavenging assay. The standard antioxidant assays were checked for percentage inhibition or scavenging activity by the formula, DPPH inhibition percentage had been calculated using the formula. DPPH inhibition (%) = $[\text{Control absorbance} - \text{Test absorbance}] \times 100$.

Table 1. Components of the high cholesterol diet administered to albino rats

Component	Measured amount (g)
Wheat flour	15
Roasted Bengal Flour	58
Groundnut Flour	10
Milk Powder	5
Powdered cashew nut	4
Salt	4
Casein	4

Table 2. Grouping of animals for experimental study *in vivo*

Groups	Experimental Design
Group I / Normal	Served as normal control rats where the diet administered was the normal diet without the cholesterol products.
Group II	Rats fed with 10g of high cholesterol diet per kg Body Weight (BW) of the rat for 45 days.
Group III	Rats fed with 10g of high cholesterol diet containing per kg of BW of the rat for 45 days along with 2mg of commercial statin per kg BW
Group IV	Rats fed with 6mg purified fungal statin from solid state fermentation in addition to 10g of high cholesterol diet per kg BW of the rat for 45 days
Group V	Rats fed with 6 mg purified fungal statin from submerged fermentation in addition to 10 g of high cholesterol diet per kg BW of the rat for 45 days

Anticholesterol analysis: Male albino rats study was performed using high cholesterol diet, where high cholesterol products were supplemented with the commercial feed (Table:1). 2mg of commercial statin tablet and 6mg of lyophilized purified fungal extract was dissolved in 1ml sterile water, was administered on all days mixing 1ml with feed. Feeding was continued for

about 45 days. After one week of adaptation, the animals were divided into 5 groups with 3 animals in each group. The control animals were fed with normal diet (Table:2). At the interval of 15days the body weight was observed. The weight at after 7days of the experiment was taken as the initial weight. After 45days, the rats were deprived with food overnight and the blood was collected by cardiac puncture. The serum was collected from blood, screened for cholesterol levels and liver marker enzymes.

Statistical analysis: The data was analysed using statistical package for social sciences (SPSS) 17.0. Mann Whitney U-test was performed to find the significance of the test results obtained as the variables were independent and the sample size was less than 10 ($n=3$). The significance value was considered at two measure of 95% ($p < 0.05$) and 99% ($p < 0.01$) were determined to be statistically significant.

RESULTS AND DISCUSSION

Aspergillus section and Flavico contains a number of different fungal species which are well known for their production of secondary metabolites. Most of these secondary metabolites are promising candidates for human use as the natural replacements for artificial chemical compounds. The objective of the study was to isolate and purify fungal statin from *Aspergillus tamarii* and determine its potency as an anticholesterol agent, antioxidant, antifungal agent and anticancer agent. In this study, we have isolated 15 fungi from various environmental sources, out of which 14 were found to be *Aspergillus flavus* and one was found to be *Aspergillus tamarii*. The aflatoxin analysis revealed that the isolated strain of *Aspergillus tamarii* was non-aflatoxigenic. Osman et al., (2011) screened twenty three fungal isolates and tested for their ability to produced lovastatin.

Screening of effects of statins on the growth of *Aspergillus fumigatus* were investigated on solidified minimal media. *Aspergillus fumigatus* exhibited robust growth with the production of conidia after 4days at 31°C. In the presence of statins, there was growth inhibition in regular *Aspergillus fumigatus* strains. Strains were initially grown on various concentrations of cholesterol with PDA and the spores were plated with statin incorporated solidified PDA. The cultures exposed to higher concentrations of cholesterol were shown higher growth in the presence of statin. (Table.3).

Antifungal activity of isolated fungal statin: The antifungal activity of the isolated fungal statin was analysed against different fungi along with commercially available statin. Antifungal susceptibility of *Candida sp.* was confirmed against the isolated fungal statin (Table: 4). Galgoczy et al., (2011) studied the *in vitro* antifungal activity of statins against yeast and filamentous fungal isolates including *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Rhizopus sp.*, *Aspergillus flavus*

and *Aspergillus fumigatus*. The inhibitory potentials of statins were studied in the range 0.25 - 128 µg/ml by broth microdilution.

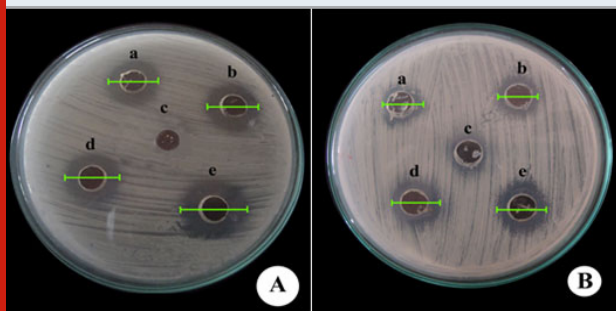
In study simvastatin (8µg/ml) displayed the strongest antifungal activity followed by fluvastatin (25- 128 µg/ml), atorvastatin (128 µg/ml), rosuvastatin (128 µg/ml) and lovastatin (5-64 µg/ml) against yeast while antifungal activity of statins against filamentous fungi showed fluvastatin (2µg/ml) to be most potent followed

by rosuvastatin (8µ/ml), simvastatin (6.25 µg/ml), lovastatin (25 µg/ml) and atorvastatin (>128 µg/ml). In our study rosuvastatin, atorvastatin, simvastatin, lovastatin, showed potency at (>100 µg/ml), while fungal statin showed inhibition at (300µg/ml) against *Candida* sp. and filamentous fungi including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus sp.*, *Excerohilum sp.* and *Fusarium sp.* did not show any inhibition against fungal statin and commercial statin.

Table 3. Bioassay using *Candida albicans* (MTCC183) showing diameter of inhibition zone using fungal extract on comparison with commercial statin

S.No.	Concentration of commercial statin (µg/ml)	Diameter of inhibition zone using commercial statin (cm)	Concentration of fungal statin (µg/ml)	Diameter of inhibition zone using fungal statin (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	Control (ethyl acetate)	-	Control (ethyl acetate)	-

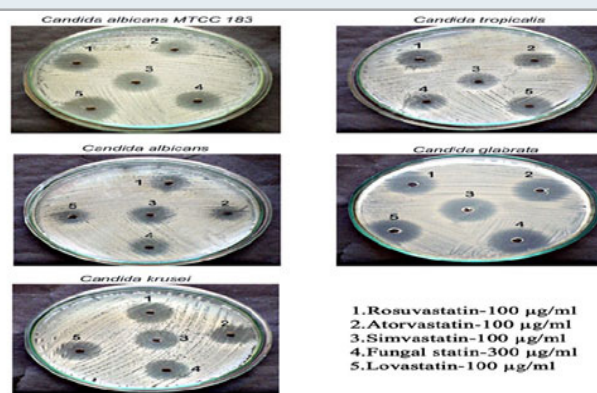
Plate 1: Diameter of zone of inhibition using *Candida albicans*(MTCC183) against(A) commercial statin and (B) fungal extract



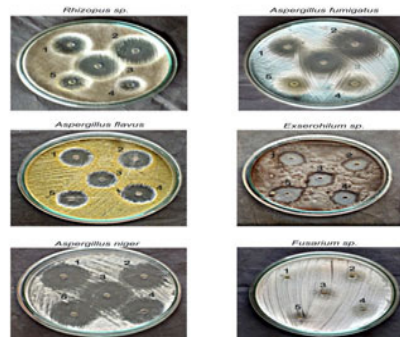
a-50 µg/ml; b-75 µg/ml; c- control (ethyl acetate); d- 100 µg/ml; e-125 µg

Antioxidant activity of isolated statin: The DPPH activity of the statin compound when compared to the commercial antioxidant BHT. The concentration dependent comparison confirmed extracted statin to possess antioxidant activity (Figure 3). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as commercial antioxidants now play a role in screening of antioxidant potential in metabolites and biopharmaceuticals extracted or isolated from plants or microbes (Ozusaglam and Karakoca, 2013). In our study, DPPH radical scavenging activity of fungal statin showed similar activity when compared to standard statin tablet although there was a significant increase in the antioxidant activity by BHT (the commercial antioxidant). The variation of the fungal statin with respect to enzymatic antioxidants is largely dose dependent. An increase in the antioxidant potential of fungal statin was

Plate 2: Antifungal activity of lovastatin and fungal statin



1. Rosuvastatin-100 µg/ml
2. Atorvastatin-100 µg/ml
3. Simvastatin-100 µg/ml
4. Fungal statin-300 µg/ml
5. Lovastatin-100 µg/ml



1 - Rosuvastatin (100 µg/ml); 2 - Atorvastatin (100 µg/ml); 3 - Simvastatin (100 µg/ml); 4 - Fungal statin (300 µg/ml); 5 - Lovastatin (100 µg/ml)

On comparison with group II, there was a significant decrease in SGOT, SGPT, and alkaline phosphatase values in group III, group IV and group V. This showed that

high fat diet had negative effect on the liver, while the total bilirubin showed only a marginal increase when compared to normal rats.

Table 5. Effect of purified statin and commercial statin on serum lipid profile of normal and induced adult male albino rats

Group	TC	TG	HDL-C	LDL-C	VLDL-C	CHO/HDL	LDL/HDL
I	62.3±1.1	56.8±3.2	26.6±1.52	24.9±1.4	11.2±0.64	2.3±0.644	0.9±0.1
II	85.8±4.7 ^a	116.6±1.2 ^a	32.6±1.5 ^b	29.5±1.44 ^b	23.5±2.6 ^a	2.62±0.03 ^a	0.9±0.5 ^b
III	68.7±1.5 ^d	46±8.7 ^c	22.6±2.51 ^c	36.8±3.40 ^c	9.2±1.74 ^d	3.05±0.38 ^d	1.6±0.3 ^c
IV	63.5±3.04 ^{ec}	34±2.64 ^{ec}	12.3±2.5 ^{dc}	39.7±1.16 ^{dc}	6.8±5.2 ^{dc}	5.26±0.87 ^{dc}	3.7±0.7 ^{dc}
V	65.01±2 ^d	41.66±4.5 ^{bd}	15.66±3.8 ^{ad}	41.01±3.8 ^{bd}	8.33±0.9 ^{ad}	4.31 ±1	2.74±0.86

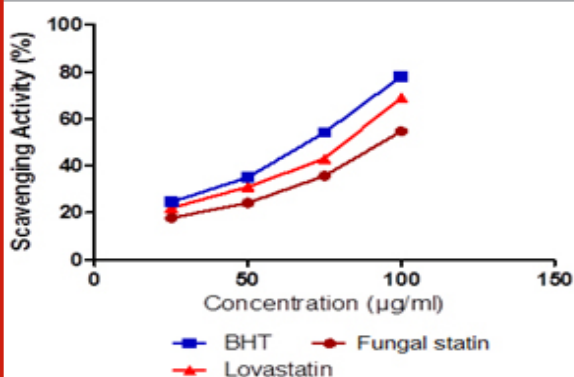
Each value is given in Mean±SEM; a - n=3 observations at p<0.01 on comparison with group I, b - n=3 observations at p<0.05 on comparison with group I, c - observations with p<0.05 on comparison with group III, d - n=3 observations at p<0.01 on comparison with group II, e -, n=3 observations at p<0.05 on comparison with group II

Table 6. Effect of purified statin obtained on submerged and solid state fermentation and commercial statin on liver marker enzymes *in vivo*

Groups	Total Bilirubin	SGOT	SGPT	Alkaline Phosphatase
I	0.3±0.1	75.4±5.1	24.8±2.5	120.25±1.93
II	1.1±0.1	86.6±7.63 ^a	61.3±3.5 ^b	175±5 ^a
III	0.4±0.1	72.4±2.25 ^c	23.5±2.21 ^c	120.3±3.06 ^c
IV	0.4±0.1	74.9±1.5 ^c	30.4±2.4 ^c	121.8±1.2 ^c
V	0.6±0.1	75.2±2.1 ^c	28.8±1.48 ^c	122.7±2.5 ^c

Each value is given in Mean±SEM; a - n=3 observations at p<0.01 on comparison with group I, b - n=3 observations at p<0.05 on comparison with group I, c- n=3 observations at p<0.01 on comparison with group II.

Figure 1: Antioxidant activity of isoalted statin



Tanideh and Badiei, (2013) studied the effect of simvastatin and garlic on lipid profile and liver marker enzymes in rats fed with normal and fat rich diet. Simvastatin significantly reduced the total cholesterol, triglycerides and LDL broth on normal diet and fat rich diet. HDL increases significantly in simvastatin treated rat. No significant changes were detected in

ALT and AST levels in different groups. Rajasekaran and Kalaivani, (2011) studied the hypolipidemic and antioxidant activity of aqueous extract of *Monascus purpureus* fermented Indian rice in high cholesterol diet fed rats. On *in vivo* evaluation of anticholesterolemic activity, the plasma total cholesterol, triglycerides, LDL and VLDL significant declined when compared to the high cholesterol fed rats. Recently Yuliana et.al (2020) have reported the fermentation and determination of Anticholesterol Monakolin K from different isolates of *Monascus purpureus*.

CONCLUSION

This is the first report on the isolation of fungal statin from *Aspergillus tamarii*. Statin is produced as a part of the polyketide pathway during fungal metabolism. Statins have a variety of biological effects which include anticholesterolemic, antimicrobial and antioxidant properties. This study was designed to isolate the fungal statin from *Aspergillus tamarii*, that has showed good potential to be used as an antifungal agent, anticancer agent, antioxidant agent and a cholesterol

lowering agent. These natural statins could be used as a replacement for the chemical drugs that come along with various side effects.

ACKNOWLEDGEMENTS

We acknowledge the management of GRD institution for providing the research funds and high class infrastructure to complete the research.

Conflict of interest: The authors declare no conflicts of interest.

REFERENCES

- Alanazi, H., Zaidan, B.B., Zaidan, A.A., Jalab, H.A., Shabbir, M. and Al-Nabhani, Y., (2010). New comparative study between DES, 3DES and AES within nine factors. arXiv preprint arXiv:1003.4085.
- Alberts, A.W., (1988). Discovery, biochemistry and biology of lovastatin. *The American journal of cardiology*, 62(15), pp.J10-J15.
- Alberts, A.W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E. and Patchett, A., (1980). Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proceedings of the National Academy of Sciences*, 77(7), pp.3957-3961.
- Arora, D.S. and Chandra, P., (2010). Assay of antioxidant potential of two *Aspergillus* isolates by different methods under various physio-chemical conditions. *Brazilian Journal of Microbiology*, 41(3), pp.765-777.
- Bedford, M.R., (1995). Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal feed science and technology*, 53(2), pp.145-155.
- Chakravarti, R. and Sahai, V., (2004). Compactin—a review. *Applied microbiology and biotechnology*, 64(5), pp.618-624.
- Chong, R.L., Sunrise Telecom Inc, (2001). Detection of bridge tap using frequency domain analysis. U.S. Patent 6,177,801.
- Endo, T., Kobayashi, S. and Onaya, T., (1985). Parvalbumin in rat cerebrum, cerebellum and retina during postnatal development. *Neuroscience letters*, 60(3), pp.279-282.
- Farnier, M. and Davignon, J., (1998). Current and future treatment of hyperlipidemia: the role of statins. *The American journal of cardiology*, 82(4), pp.3J-10J.
- Franzoni, F., Quiñones-Galvan, A., Regoli, F., Ferrannini, E. and Galetta, F., (2003). A comparative study of the in vitro antioxidant activity of statins. *International journal of cardiology*, 90(2-3), pp.317-321.
- Furberg, C.D., Herrington, D.M. and Psaty, B.M., (1999). Are drugs within a class interchangeable?. *The Lancet*, 354(9185), pp.1202-1204.
- Galgóczy, L.N., Nyilasi, I., Papp, T. and Vágvölgyi, C., (2011). Statins as antifungal agents. *World Journal of Clinical Infectious Diseases*, 1(1), pp.4-10.
- Galgóczy, L.N., Nyilasi, I., Papp, T. and Vágvölgyi, C., (2011). Statins as antifungal agents. *World Journal of Clinical Infectious Diseases*, 1(1), pp.4-10.
- Jenkins, S.P., (2005). Survival analysis. Unpublished manuscript, Institute for Social and Economic Research, University of Essex, Colchester, UK, 42, pp.54-56.
- Kalaivani, T., Rajasekaran, C., & Mathew, L. (2011). Free radical scavenging, cytotoxic, and hemolytic activities of an active antioxidant compound ethyl gallate from leaves of *Acacia nilotica* (L.) wild. Ex. Delile subsp. *Indica* (Benth.) Brenan. *Journal of food science*, 76(6), T144-T149.
- Karakoca, K., Ozusaglam, M.A., Cakmak, Y.S. and Erkul, S.K., (2013). Antioxidative, antimicrobial and cytotoxic properties of *Isatis floribunda* Boiss. exBornm. extracts. *EXCLI journal*, 12, p.150.
- Maron, D.J., Fazio, S. and Linton, M.F., (2000). Current perspectives on statins. *Circulation*, 101(2), pp.207-213.
- Mehra, A., Narang, R., Jain, V.K. and Nagpal, S., (2020). Preparation and characterization of nano statins using oyster mushroom (*Pleurotuss ajorcaju*): a new strategy to reduce toxicity and enhance efficacy for the treatment of cardiovascular disease. *European Journal of Integrative Medicine*, 33, p.101014.
- Morris, J.C., Eastman Kodak Co, (1993). Polyester/polycarbonate blends. U.S. Patent 5,239,020.
- Osman, S.O., Moustafa, F.M.A., Abd El-Galil, H.A. and Ahmed, A.Y.M., (2011). Effect of yeast and Effective Microorganisms (Em1) application on the yield and fruit characteristics of Bartamuda date palm under Aswan conditions. *Assiut J. of Agric. Sci*, 42(5), pp.332-349.
- Srinu, S.L.S.S. and Sabat, S.L., (2010), October. FPGA implementation of spectrum sensing based on energy detection for cognitive radio. In 2010 International Conference on Communication Control And Computing Technologies (pp. 126-131). IEEE.
- Stein, L.H., Stefferud, E.A., Borenstein, N.S. and Rose, M.T., First Virtual Holdings Inc, (1998). Computerized system for making payments and authenticating transactions over the internet. U.S. Patent 5,826,241.
- Subhan, M., Faryal, R. and Macreadie, I., (2016). Exploitation of *Aspergillus terreus* for the Production of Natural Statins. *Journal of Fungi*, 2(2), p.13.
- Tanideh, N. and Badiei, R., (2013). Evaluation of the effects of simvastatin alone and in combination with garlic on lipid profile and liver enzymes in rats fed normal and fat rich diet. *Middle-East Journal of Scientific Research*, 15(9), pp.1237-41.

Tavakkoli, A., Johnston, T.P. and Sahebkar, A., (2020). Antifungal effects of statins. *Pharmacology & Therapeutics*, 208, p.107483.

Tobert, J.A., (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nature reviews Drug discovery*, 2(7), pp.517-526.

Varga, S.M. and Spaulding, C.J., Procter and Gamble Co, (2011). Systems And Methods For Monitoring On-Line Webs Using Line Scan Cameras. U.S. Patent Application 12/639,266.

Wassman, F., (2002). Green Solutions. Jakob AG,

Trubschachen, Switzerland.

Yuliana, A., Zannah, S.U., Rahmiyani, I. and Amin, S., (2020), June. Fermentation and Determination of Anti-Cholesterol Monakolin K from Different *Monascus purpureus* Isolates. In 2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019) (pp. 225-227). Atlantis Press.

Zhang, Y., Chen, Z., Wen, Q., Xiong, Z., Cao, X., Zheng, Z., Zhang, Y. and Huang, Z., (2020). An overview on the biosynthesis and metabolic regulation of monacolin K/lovastatin. *Food & Function*.