Biomedicine and Chemical Sciences 2(2) (2023) 155-162

14-BCS-1565-350

Content lists available at:
www.journals.irapa.org/index.php/BCS/issue/view/23

Biomedicine and Chemical Sciences

Journal homepage: https://www.journals.irapa.org/index.php/BCS

Optimization and Characterization of Flavipin Produced by *Aspergillus Terreus*

Mohammed Jasim Qasim[1]*, Azhar A. F. Al-Attraqchi[1] & Gazi M. Aziz[2]

1 Department of Microbiology, College of Medicine, Al-Nahrain University – Iraq
2 Department of Biotechnology, College of Science, Baghdad University – Iraq

ARTICLEINFO

Article history:
Received on: November 28, 2022
Revised on: December 28, 2022
Accepted on: February 02, 2023
Published on: April 01, 2023

Keywords:
Aspergillus terreus
Flavipin
HPLC
Optimal conditions
TLC

ABSTRACT

The secondary metabolites of microorganisms serve as defence or signalling molecules in ecological interactions, revealing substantial survival benefits in nature. As a result, many researchers have concentrated on screening and optimizing the production of these molecules from natural sources such as microorganisms with the objective of pharmacological uses, primarily as antibiotics or anticancer agents**.** In this study, 80 isolates of Aspergillus were investigated for the production of flavipin. These fungi were collected from various locations and laboratories. Flavipin was estimated by using a standard curve, then purified by using silica gel chromatography, followed by identification using thin layer chromatography (TLC), and High Performance liquid chromatography (HPLC). The fermentation conditions were carried out at the Central Health Laboratory/Maysan Health Directorate from April 2021 to August 2022. Out of eighty isolates of Aspergillus, only one isolate was identified as producer of flavipin which was *Aspergillus terreus*. According to HPLC analysis, the retention times of flavipin and its standard were 7.7 minutes and 7.6 minutes, respectively. By using the TLC technique, the relative flow (Rf) value was 0.55 cm for both standard flavipin and flavipin. The optimization of growth conditions and production of flavipin were studied. It is revealed that optimum conditions were as follows: pH 7 on 16 days, the temperature of 25°C for 12 days, culture volume of 50 ml on the 16th day, shaking speed of 150 rpm on the 12th day, inoculum size of 8 fungal agar disc on the 12th day, the optimal incubation period of 14 days, and Potato Dextrose Broth as the optimal culture media. The aim of the study was to determination of optimal conditions for the flavipin production that produced by *Aspergillus terreus*. For yielding a profuse amount of flavipin, the incubation and fermentation conditions such as temperature, the culture volume, shaking speed, inoculum size, pH of the medium, incubation period, and the type of culture media should be considered and the optimal one must be chosen.

1. Introduction

Secondary metabolites isolated from Aspergillus have constantly attracted the attention of pharmacologists because of their broad range of biochemical activities and their unique properties (Cai, et al., 2011; Wang, et al., 2017). Aspergillus has been shown to be a prolific producer of secondary metabolites with interesting biological properties, including antibiotic activity (Wang, et al., 2015). Previous studies demonstrated that flavipin can be produced by *Aspergillus tereus* and *Aspergillus flavus* (Raistrick & Rudman, 1956; Gehlot & Singh, 2018; Lloyd, 2012). Other studies showed that flavipin could also be abstracted by *Aspergillus fumigatus* and *Aspergillus flavipes* (Flewelling, et al., 2015; Pettersson, 1965). Furthermore, flavipin can be produced by *Chaetomium globosum*, *Chaetomium globosum* CDW7, and *Epicoccum nigrum* (Kumar, et al., 2019; Xiao, et al., 2013; Ye, et al., 2013; Yan, et al., 2018; Burge, et al., 1976; Madrigal, et al., 1991; Bamford, et al., 1961).

***Corresponding author:** Mohammed Jasim Qasim, Department of Microbiology, College of Medicine, Al-Nahrain University – Iraq

E-mail: **mohammed_j82qasim@yahoo.com**

How to cite:

Qasim, M. J., Al-Attraqchi, A. A. F., & Aziz, G. M. (2023). Optimization and Characterization of Flavipin Produced by Aspergillus Terreus. *Biomedicine and Chemical Sciences, 2*(2), 155–162.

DOI: **https://doi.org/10.48112/bcs.v2i2.350**

Raistrick et al, (1965) showed that flavipin, has an empirical formula C9H8O5, sublimes easily and melts at 233-234°C. This compound is optically inactive, with maximum UV absorption of around 261, 264. Flavipin's structure has been determined to be 1, 2-diformy1-4, 5, 6-trihydroxy-3-methylbenzene. It is a member of the same natural compound as gladiolic acid and cyclopaldic acid. All of these compounds are substituted phthalic aldehydes in structure. Flavipin slowed the high antimicrobial activity against the growth of *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) (Flewelling, et al., 2015). Flavipin appears to be very powerful against some Chlorella species, while it has a mild inhibitory effect against *Bacillus megaterium*. Flavipin also appears to have phytotoxic properties (Burge, et al., 1976). It has two aldehyde groups and three phenolic hydroxyls, which were antioxidant-active functional groups. However, the antioxidant action of flavipin has received little attention (Mapari, et al., 2005). Ye et al (2013) reported the screening and optimization of endophytic fungi isolated from Ginkgo biloba for a highly yielded bio-source of flavipin.

The present study aims to optimize and demonstrate the effect of different factors and factor–factor interactions on flavipin production by isolated *Aspergillus terreus* using different conditions of fermentation.

2. Materials and Methods

Collection of isolates

Eighty species of Aspergillus were used in this study to determine which one of these isolates was producer of flavipin. These fungi were isolated from different sources such as air, water, plants (endophytic fungi), and soil. As well as some species were received from the Central Health Laboratory in Maysan Health Directorate, the Microbiology Department/College of Medicine/Misan University, and the Biology Department/College of Science/Misan University. This study was achieved from April 2021 to August 2022.

Morphological and Molecular Identification of Aspergillus Species

The morphology of colonies, and microscopic features were tested according to criteria mentioned by M De La Maza (1997) and Ellis et al (2007). The extraction of DNA from fungi was carried out using the method described by Alshehri and Palanisamy (2020) and the steps were done according to the kit (Presto™ Mini gDNA Yeast Kit/ Genaid/ USA). Universal primers for ITS1 and ITS2 were used for molecular identification in the present study. The NCBI/BLAST search program was used to find the most closely related sequences in the GenBank database.

Screening the Fungal Isolates for Flavipin Production

Spore suspensions of each Aspergillus isolate were prepared by collecting spores from 5-day old colonies grown on Potato Dextrose Agar (HiMedia, India) at 25°C. Conidia were harvested from sub-cultures in an aqueous solution of Tween 80 (0.1%) (Merck Germany) by scraping the surface of the mycelium. The final concentration of spores was assessed by a Neubauer counting chamber (hemocytometer) and adjusted by appropriate dilutions to 10^6 spores/ ml.

Fermentation and Extraction

Fungal isolates were grown on a PDA medium for 5 to 10 days at 25 °C (potato, 200 g; dextrose, 20 g; agar, 20 g; H2O, 1L). Eight pieces (5 mm in diameter) of mycelial agar plugs taken from the edge of early endophytic fungus cultures were inoculated into each flask (250-mL Erlenmeyer flask) with 200 mL of PD broth medium, followed by continuous shaking (150 rpm) at 25 ±1 °C for 12 days. To separate the culture broth and mycelia, the broth culture was filtered by using Whatman (No. 1) filter paper. Ethyl acetate (1mL) was used three times to extract the culture broth. The extracted ethyl acetate was mixed and dried with a rotary evaporator (BUChI, Swiss). Until analysis, all extracts were kept at 4 °C (Flewelling, et al., 2015; Ye, et al., 2013).

Purification of Flavipin

The purification of flavipin from the producer species was achieved using silica gel column chromatography (Merck, USA). In the oven at 90-120 °C for 24 hours, the 25 gm of silica (60-120 mesh) was activated , then suspended in 25 mL of methanol and put tightly into the column chromatography (36 x 1.5 cm size) without air bubbles. The extract of flavipin was concentrated at room temperature for 24 hours before being placed on silica gel column chromatography using dichloromethane and ethyl acetate mixture (v/v, 1:1) as solvents. A total of 13 mL of flavipin extract was gently passed throughout the column using a pipette (a pasture pipette). The crude extract was eluted with an ethyl acetate and dichloromethane combination at a rate of 20 mL/hour, and four fractions were obtained.

Estimation of Flavipin

Flavipin was determined using the standard curve method as described by Ye, et al. (2013). The standard curves were created by serially diluting flavipin (0, 20, 40, 60, 80, 100, and 120 µg/ml). A UV-visible spectrophotometer was used to estimate its optical density at 264 nm. A standard curve was plotted by graphing absorbency vs standard concentrations. The unknown flavipin concentrations in samples were determined using the equation Y = 0.032X, where Y is the absorbance or optical density obtained with a UV-Visible Spectrophotometer and X is the flavipin concentrations (Ye, et al., 2013).

Detection of Flavipin

For detection of the purity of flavipin, the thin Layer Chromatography (TLC) test was used to determine the relative flow (Rf) of both flavipin and standard flavipin using the following formula:

Rf = compound travel distance in centimeters / solvent travel distance in centimeters. Additionally, high-Performance Liquid Chromatography (HPLC) was also used to detect the retention time (Rt) for flavipin and the standard flavipin. The concentration of flavipin was detected depending on the following equation:

Concentration of flavipin = area of peak (flavipin)/area of peak (standard flavipin) x concentration of standard.

Optimization of Fermentation Conditions

Four main cultures of Sabouraud's Dextrose Broth (Oxoid, England), Potato Dextrose Broth (Locally synthesized), oat

bran medium (Locally synthesized), and malt extract broth (Locally synthesized) were selected to optimize the culture conditions based on ethyl acetate extraction. Temperatures (21°C, 25°C, 29°C, and 33°C), culture volumes (25, 50, 100, and 150 mL in a 250-mL flask), shaking speed (120, 150, 180, and 210 rpm), fermentation time (8-20 days, every 2 days), and pH were all investigated. Because time was one of the most important factors for metabolites production and since several factors chosen for this research, such as inoculum size and culture volumes, interacted with each other, the method of time tracking was used, which included sampling from flasks with various factors on the 16th, 12th, and 8th days to optimize the parameters of fermentation for higher production of flavipin (Ye, et al., 2013).

3. Results and Discussion

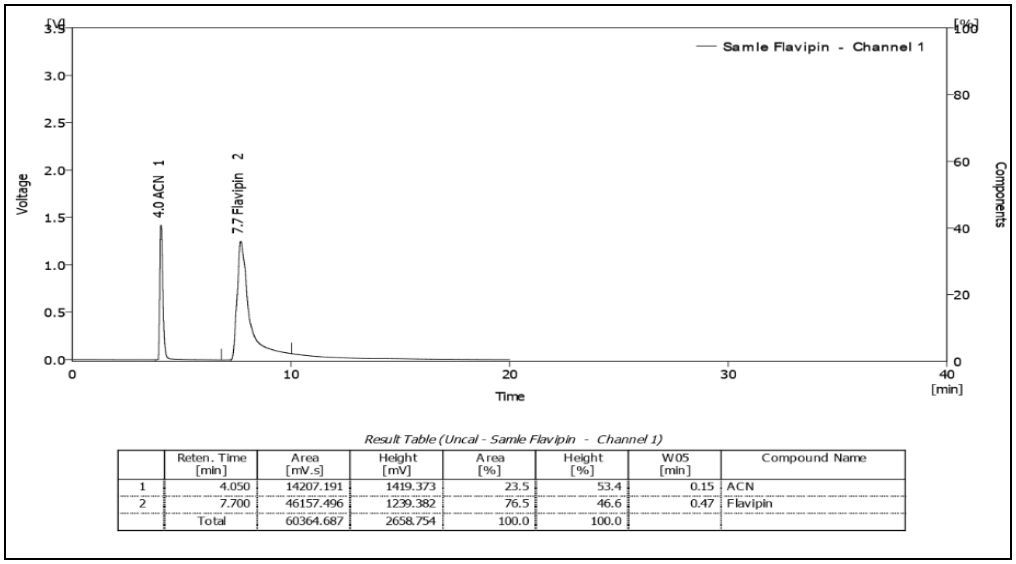
Out of 80 isolates of *Aspergillus spp.*, only one was seen to be flavipin producers which was *Aspergillus terreus*.

Aspergillus terreus colonies varied in color from buff to beige to cinnamon in culture. When stained with Lactophenol Cotton Blue (HiMedia / India), conidia looked globose and tiny (2-2.5 m). Conidial heads were columnar and biseriate with hyaline and smooth-walled. Regarding the morphological study of *A. terreus*, it was found that the colonies varied in color from buff to cinnamon in culture and the conidia looked globose and tiny. Conidial heads were columnar and biseriate with hyaline and smooth-walled. These results were in agreement with the studies of Upendra et al., (2013), Arora et al., (2021), and Attia, et al., 2022. They found that *Aspergillus terreus* colonies on PDA plates were cinnamon brown, conidiophores were biseriated with smooth-walled conidiophores, and 8-12 mm pyriform vesicles contained hyaline tiny, globose, and smooth conidia.

The sequencing of producer *Aspergillus terreus* was 98.82% identical to that of *Aspergillus terreus* strain CFE-142, and it was assigned the accession number MN686501.1. The sequence of *A. terreus* had a higher similarity (98.82%) with *A.terreus* strain CFE-142, in which the E. value was 0.0 and the accession number was MN686501.1. Depending on NCBI/BLAST data, *A. terreus* strain CFE-142.

Flavipin, the standard, and the crude were spotted on TLC plates after purification procedures were used to estimate the Rf value of flavipin by comparing it to the standard. After applying the compounds to the plate, the mobile phase (solvent mixture) was moved up the plate by capillary action, and the spots were visible by shining ultraviolet light onto the sheet to determine their positions. This may be accomplished simply by shining ultraviolet light over the sheet. The Rf value for both compounds was 0.55 cm.

The HPLC method was used to determine the quantity and quality of the sample (flavipin) as well as the standards. The retention time (Rt) of the examined sample was obtained and compared to the Rt of the standard to establish qualitative identification. The HPLC results are shown in Figure (1) including Rt and peak area.

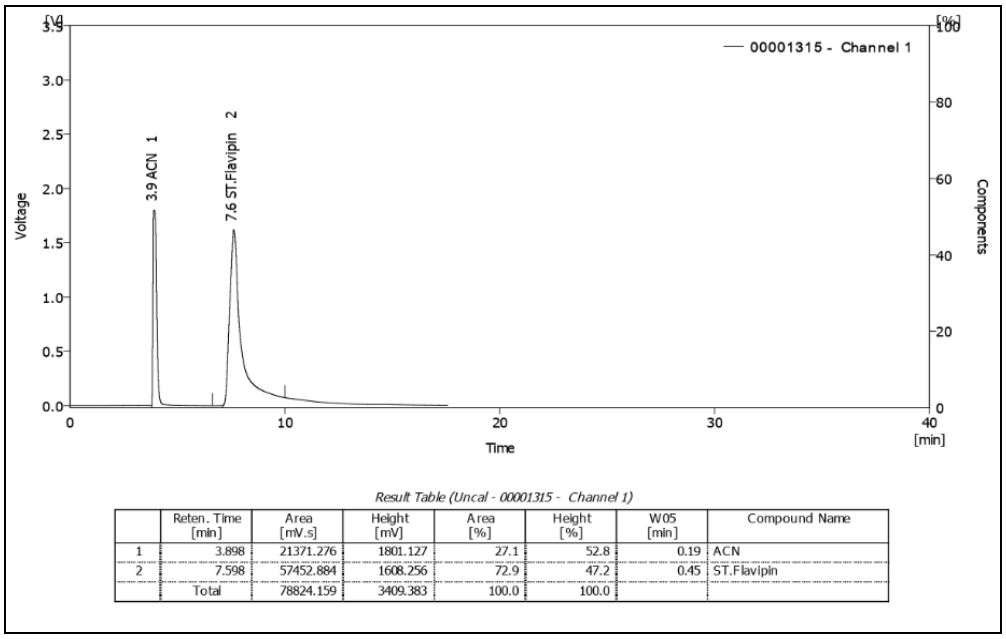


Result Table (Uncal - Sample Flavipin - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.050	14207.191	1419.373	23.5	53.4	0.15	ACN
2	7.700	46157.496	1239.382	76.5	46.6	0.47	Flavipin
	Total	60364.687	2658.754	100.0	100.0		

Fig. 1. Demonstrates HPLC analysis of flavipin produced from local isolate *Aspergillus terreus*

The retention time and peak area of the standard were 7.598 min. (about 7.600) and 57452.884 mV.s, respectively, according to (figure 2). The mobile phase in both flavipin and its standard was acetonitrile (ACN), with retention times of 4.050 and 3.898 minutes, respectively.



Result Table (Uncal - 00001315 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.898	21371.276	1801.127	27.1	52.8	0.19	ACN
2	7.598	57452.884	1608.256	72.9	47.2	0.45	ST.Flavipin
	Total	78824.159	3409.383	100.0	100.0		

Fig. 2. Demonstrates HPLC analysis of standard flavipin produced from local isolate *Aspergillus terreus*

According to figure 1 and 2, the retention times of flavipin and its standard were 7.700 minutes and 7.598 minutes, respectively. These results were approximately similar to the results of Ye et al. (2013). They showed that the retention time of flavipin was 7.97 minutes, while retention times according to the study of Flewelling et al, (2015) and Madrigal et al. (1993) were 15.19 minutes and 2.3 minutes respectively. These differences in results may be because of the difference in conditions and specifications of their works such as types of device protocol, temperature, and solvents that were used in each study. This explains supported by the study of Stanstrup et al, (2015) and Naylor et al, (2020), they concluded that the variances in HPLC systems, columns, eluents, and gradients could result in obvious differences in the retention time of the same substance under various conditions. This is especially true when conducted by several labs, even when the same HPLC system and chromatography column are used.

The purification of flavipin was assessed by calculation of the Rf value of each standard flavipin and flavipin. It was 0.55 cm for both. This result disagreed with the study of Kumar et al. (2019) which showed that the Rf value of flavipin was 0.43 cm. Another study showed that the Rf of flavipin was 0.69 cm (Pettersson, 1965). The current study was in agreement with the previous studies which revealed that the Rf value of flavipin was 0.55 cm (Madrigal, et al., 1991; Madrigal, et al., 1993).

The Rf values can be affected by a number of different factors, such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the plate, and solvent parameters.

Optimization of Flavipin Fermentation Condition

The Effect of pH on Flavipin Production

From the plotted results, it can be observed that pH value 7 exhibited the maximum productivity of flavipin (8.25 µg/ml) in day12 of incubation. The lower levels of production that were obtained from cultures with a pH value of 9 on the days 8, 12, and 16 which were 2.28 µg/ml, 2.44 µg/ml, and 2.12 µg/ml, respectively (figure 3). Statistical analysis reveals that there was a significant difference ($p = 0.007$) among the different products of flavipin regarding various pH values.

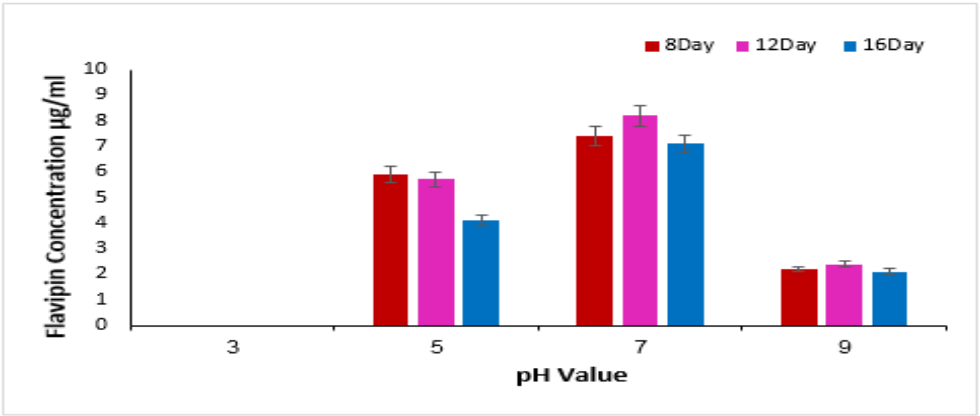


Fig. 3. Flavipin concentrations with different pH value in various incubation periods

The Factor of Culture Volume per a Flask

Figure (4) presents flavipin production under different culture volumes (25, 50, 100, and 150 ml/flask of 250 ml) which were incubated for different periods (8 days, 12 days, and 16 days). The concentration of flavipin with volume of 50 ml/flask in the 16 days was 10.91 µg/ml which represented the optimal volume. It was higher than other volumes at different incubation days.

There were highly significant differences between the concentrations of flavipin under different incubation volumes (*p*-Value= 0.000).

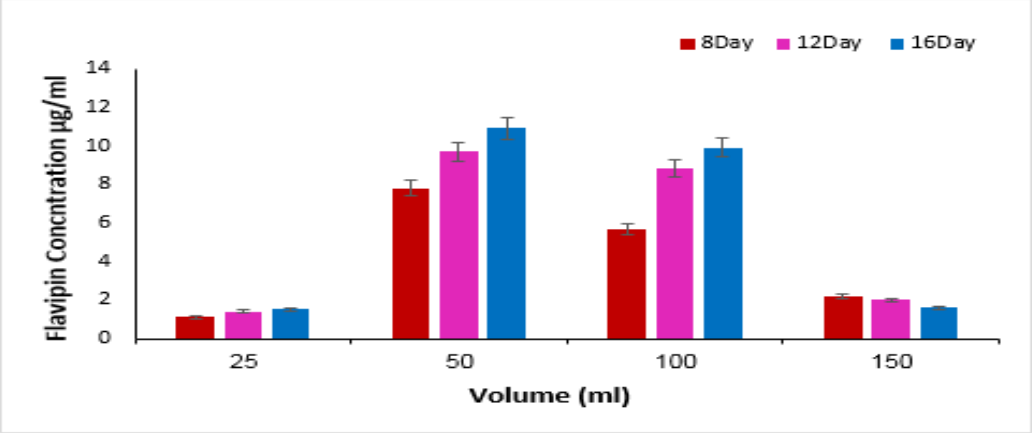


Fig. 4. Flavipin concentration in different periods of incubation and different volumes of broth

Temperature Factor

According to the results, flavipin concentration was 14.26 µg/ml after 12 days of shaking incubation at 25 °C, which was the optimal temperature for productivity. The lowest amount of production occurred after 16 days of incubation at 21 °C (figure 5). By using One-Way Analysis of Variance (ANOVA), statistical analysis showed highly significant differences between the results of the production under different selected temperatures in the present study (p = 0.000).

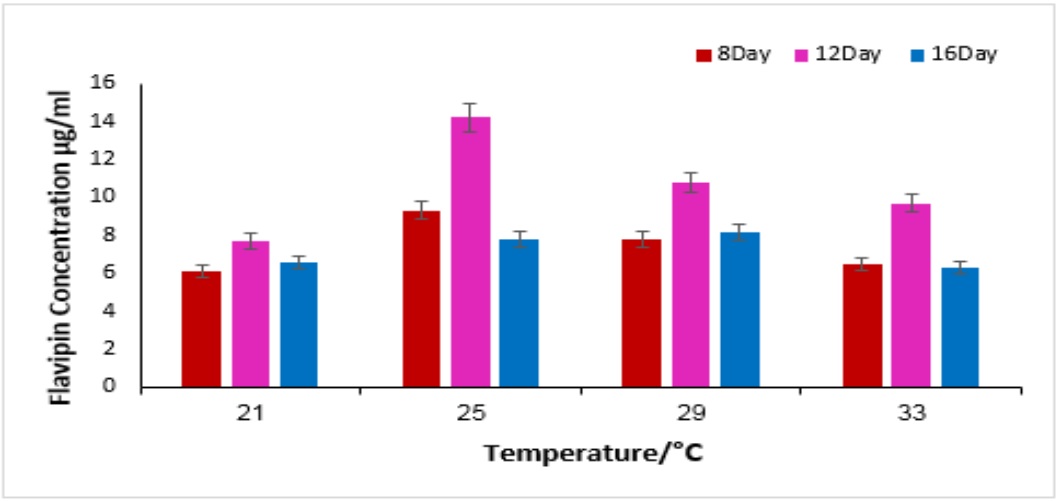


Fig. 5. Shows the effect of temperature on flavipin production

Shaking Speed of Incubation

The results showed that a shaking speed of 150 rpm on the 12th day was a typical condition for flavipin production (15.16 µg/ml). Statistically, there was a significant difference (p = 0.003) between the concentration results of different speeds of shaking broth (figure 6).

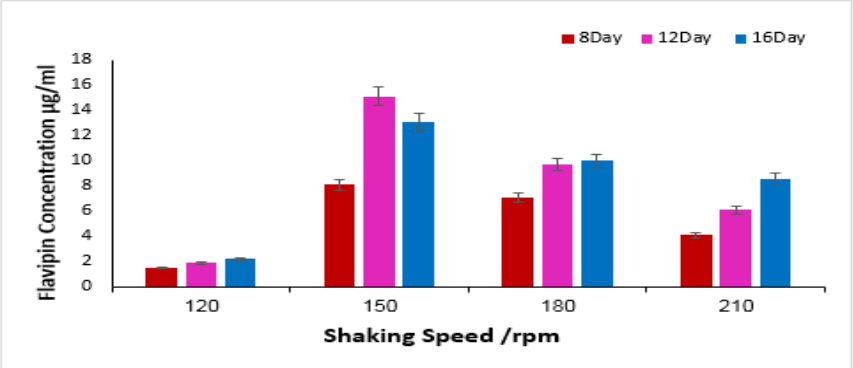


Fig. 6. Demonstrates the effect of shaking speed on flavipin production

The Effect of Inoculum Size on Flavipin Production

According to figure (7), the higher concentration of flavipin was 15.82 µg/ml. This result obtained when the eight fungal agar discs (inoculum size) were inoculated in each flask for 12 days of incubation which represented the optimal conditions for flavipin production regarding the factor of inoculum size, while productivity began to decline when the broth was inoculated with 12 and 16 agar discs. Statistical analysis revealed a significant differences in production outcomes based on the different inoculum sizes ($p = 0.003$).

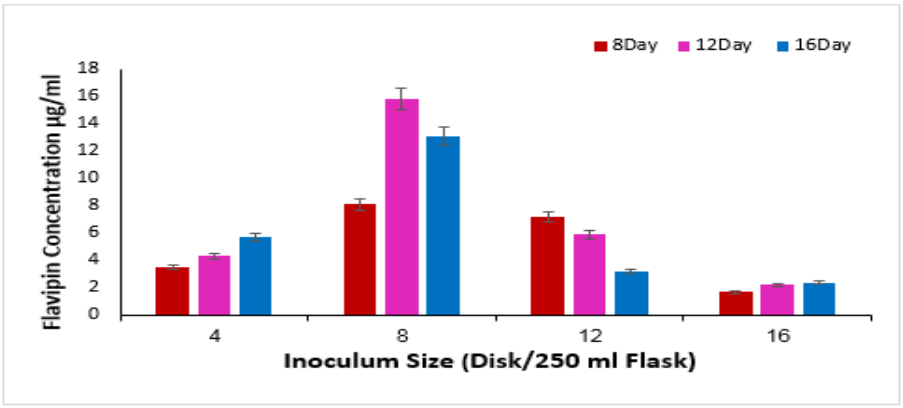


Fig. 7. The flavipin production with different inoculum size (disk/250 ml flask)

The Effect of Incubation Period

As results are shown in figure 8, there was a progressive increase in flavipin production from day 8 to day 14 of incubation. The optimum day of incubation was 14, in which the *Aspergillus terreus* produced the highest yield of flavipin which was 16.32 µg/ml. Then, flavipin productivity began to decrease when the broth was incubated for 16, 18, and 20 day sequentially.

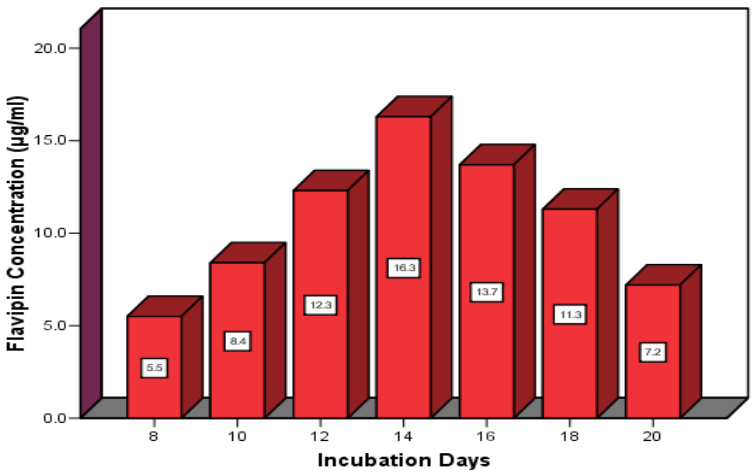


Fig. 8. Demonstration of flavipin production in different incubation days

The Effect of Culture Media

The effects of various culture media on the production of flavipin were investigated (figure 9). Potato Dextrose Broth (PDB) was found to be the most effective medium compared to other media which were Sabouraud's Dextrose Broth (SDB), Malt Extract.

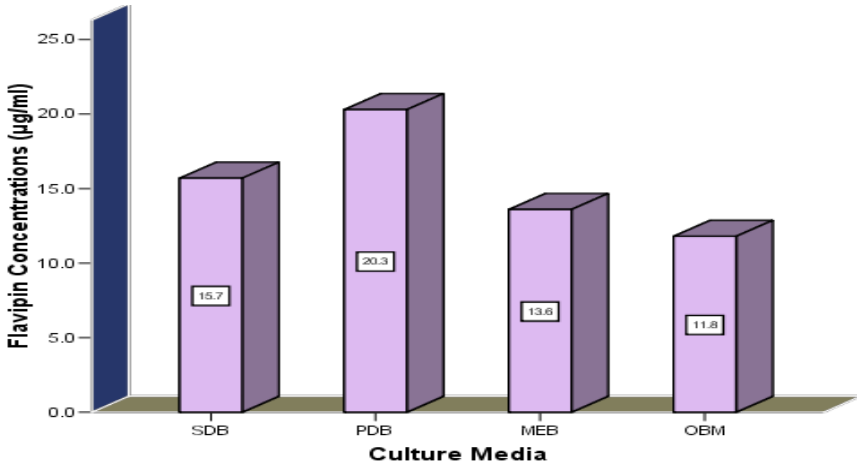


Fig. 9. Flavipin production with different culture media

Broth (MEB), and Oat Bran Medium (OBM). The highest flavipin concentration obtained on PDB was 20.3 µg/ml, while the lowest productivity of flavipin was 11.8 µg/ml, which was yielded from OBM as solid state fermentation. Regarding SDB and MEB, which were represented as submerged fermentation, the produced concentrations were 15.7 µg/ml and 13.6 µg/ml, respectively.

In the current study, the results displayed an effect of different incubation conditions and demonstrated the optimal one for the production of flavipin from *Aspergillus terreus*. In most parameters, the results were consistent with those of Ye et al. (2013), who discovered that the PD medium was the best of the four tested media and that 25 °C was chosen as the optimum culture temperature, as well as that 50 ml/250-mL flask on the 16th day performed slightly better than 100 ml. Furthermore, the shaking speed of 150 rpm was significantly better than the other speeds, and 14 days of fermentation was accounted as the best processing time, despite the fact that they only yielded higher flavipin in pH 6.5. Furthermore, this study was in agreement with the results of Xiao et al. (2013) in using the same fermentation conditions for flavipin production. They used eight pieces of mycelial agar removed from the edges of young cultures and placed them in 100 ml of PD broth per 250-mL Erlenmeyer flask, followed by shaking (150 rpm) continuously for 12 days at 25 °C. Flewelling et al. (2015), also used the same optimal conditions in some points for the production of flavipin from *Aspergillus fumigatus* by using malt extract broth with a volume of 100 mL in 250 mL Erlenmeyer flasks at 25 °C for 14 days with shaking (150 rpm).

4. Conclusion

According to the above results, It has been concluded that the optimal conditions for flavipin production regarding temperature, the volume of broth, shaking speed, inoculum size, pH of medium, incubation period, and the type of culture media were 25 °C for 12 days, 50 ml on the 16th day, 150 rpm on the 12th day, 8 fungal agar discs on the 12th day, 7 pH in 16 days, 14 days, and Potato Dextrose Broth, respectively.

Acknowledgement

The authors are very grateful to all academic members and technician workers for their assistance to complete this study.

Competing Interests

The authors have declared that no competing interests exist.

References

Alshehri, B., & Palanisamy, M. (2020). Evaluation of molecular identification of Aspergillus species causing fungal keratitis. Saudi Journal of Biological Sciences, 27(2), 751-756. https://doi.org/10.1016/j.sjbs.2019.12.030

Arora, P., Kumar, A., A Vishwakarma, R., & Riyaz-Ul-Hassan, S. (2021). A natural association of a yeast with Aspergillus terreus and its impact on the host fungal biology. FEMS Microbiology Letters, 368(6), fnab032. https://doi.org/10.1093/femsle/fnab032

Attia, M. S., Hashem, A. H., Badawy, A. A., & Abdelaziz, A. M. (2022). Biocontrol of early blight disease of eggplant using endophytic Aspergillus terreus: improving plant immunological, physiological and antifungal activities. Botanical Studies, 63(1), 26. https://doi.org/10.1186/s40529-022-00357-6

Bamford, P. C., Norris, G. L. F., & Ward, G. (1961). Flavipin production by Epicoccum spp. Transactions of the British Mycological Society, 44(3), 354-356. https://doi.org/10.1016/S0007-1536(61)80028-4

Burge, W. R., Buckley, L. J., Sullivan Jr, J. D., McGrattan, C. J., & Ikawa, M. (1976). Isolation and biological

activity of the pigments of the mold Epicoccum nigrum. Journal of Agricultural and Food Chemistry, 24(3), 555-559. https://doi.org/10.1021/jf60205a005

Cai, S., Sun, S., Zhou, H., Kong, X., Zhu, T., Li, D., & Gu, Q. (2011). Prenylated polyhydroxy-p-terphenyls from Aspergillus taichungensis ZHN-7-07. Journal of natural products, 74(5), 1106-1110. https://doi.org/10.1021/np2000478

Ellis, D. H., Davis, S., Alexiou, H., Handke, R., & Bartley, R. (2007). Descriptions of medical fungi (Vol. 2). Adelaide: University of Adelaide.

Flewelling, A. J., Bishop, A. L., Johnson, J. A., & Gray, C. A. (2015). Polyketides from an endophytic Aspergillus fumigatus isolate inhibit the growth of Mycobacterium tuberculosis and MRSA. Natural product communications, 10(10). https://doi.org/10.1177/1934578X1501001009

Gehlot, P., & Singh, J. (Eds.). (2018). Fungi and their role in sustainable development: current perspectives. Singapore: Springer Singapore.

Kumar, V. S., Kumaresan, S., Tamizh, M. M., Islam, M. I. H., & Thirugnanasambantham, K. (2019). Anticancer potential of NF-κB targeting apoptotic molecule "flavipin" isolated from endophytic Chaetomium globosum. Phytomedicine, 61, 152830. https://doi.org/10.1016/j.phymed.2019.152830

Lloyd, D. (2012). Topics in Carbocyclic Chemistry: Volume One. Springer Science & Business Media.

M De La Maza, L. (1997). Color Atlas of diagnostic microbiology. Mosby.

Madrigal, C., Tadeo, J. L., & Melgarejo, P. (1991). Relationship between flavipin production by Epicoccum nigrum and antagonism against Monilinia laxa. Mycological Research, 95(12), 1375-1381. https://doi.org/10.1016/S0953-7562(09)80388-2

Madrigal, C., Tadeo, J. L., & Melgarejo, P. (1993). Degradation of flavipin by Monilinia laxa. Mycological Research, 97(5), 634-636. https://doi.org/10.1016/S0953-7562(09)81189-1

Mapari, S. A., Nielsen, K. F., Larsen, T. O., Frisvad, J. C., Meyer, A. S., & Thrane, U. (2005). Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants. Current Opinion in Biotechnology, 16(2), 231-238. https://doi.org/10.1016/j.copbio.2005.03.004

Naylor, B. C., Catrow, J. L., Maschek, J. A., & Cox, J. E. (2020). QSRR automator: A tool for automating retention time prediction in lipidomics and metabolomics. Metabolites, 10(6), 237. https://doi.org/10.3390/metabo10060237

Pettersson, G. (1965). The biosynthesis of flavipin. II. Incorporation of aromatic precursors. Acta chemica Scandinavica, 19(7), 1724-1732. https://doi.org/10.3891/acta.chem.scand.19-1724

Raistrick, H., & Rudman, P. (1956). Studies in the biochemistry of micro-organisms. 97. Flavipin, a crystalline metabolite of Aspergillus flavipes (Bainier & Sartory) Thom & Church and Aspergillus terreus Thom. Biochemical Journal, 63(3), 395. https://doi.org/10.1042%2Fbj0630395

Stanstrup, J., Neumann, S., & Vrhovsek, U. (2015). PredRet: prediction of retention time by direct mapping between multiple chromatographic systems. Analytical chemistry, 87(18), 9421-9428. https://doi.org/10.1021/acs.analchem.5b02287

Upendra, R. S., Pratima, K., Amiri, Z. R., Shwetha, L., & Ausim, M. (2013). Screening and molecular characterization of natural fungal isolates producing lovastatin. J Microb Biochem Technol, 5(2), 25-30.

Wang, W., Liao, Y., Tang, C., Huang, X., Luo, Z., Chen, J., & Cai, P. (2017). Cytotoxic and antibacterial compounds from the coral-derived fungus Aspergillus tritici SP2-8-1. Marine Drugs, 15(11), 348. https://doi.org/10.3390/md15110348

Wang, Y. T., Xue, Y. R., & Liu, C. H. (2015). A brief review of bioactive metabolites derived from deep-sea fungi. Marine drugs, 13(8), 4594-4616. https://doi.org/10.3390/md13084594

Xiao, Y., Li, H. X., Li, C., Wang, J. X., Li, J., Wang, M. H., & Ye, Y. H. (2013). Antifungal screening of endophytic fungi from Ginkgo biloba for discovery of potent anti-phytopathogenic fungicides. FEMS microbiology letters, 339(2), 130-136. https://doi.org/10.1111/1574-6968.12065

Yan, W., Cao, L. L., Zhang, Y. Y., Zhao, R., Zhao, S. S., Khan, B., & Ye, Y. H. (2018). New metabolites from endophytic fungus Chaetomium globosum CDW7. Molecules, 23(11), 2873. https://doi.org/10.3390/molecules23112873

Ye, Y., Xiao, Y., Ma, L., Li, H., Xie, Z., Wang, M., ... & Liu, J. (2013). Flavipin in Chaetomium globosum CDW7, an endophytic fungus from Ginkgo biloba, contributes to antioxidant activity. Applied microbiology and biotechnology, 97, 7131-7139. https://doi.org/10.1007/s00253-013-5013-8