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Research Article

Optimal conditions for the high yield of bioactive asterric acid from marine derived Fungus Aspergillus sp.

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Abstract

Marine-derived microorganisms are vital sources of bioactive secondary metabolites. Secondary metabolites of coral-derived fungi have several essential bioactivities. Asterric acid 1 is a broad-spectrum bioactive secondary metabolite of several fungal genera, especially Aspergillus and Penicillium. It has a wide range of interesting bioactivities such as antifungal, antimicrobial, cytotoxic, fungicidal, pesticidal, nematicidal, seed germination inhibition, anti-HIV activity, as a first non-peptide for endothelin binding inhibition, anti-plasmodial activity against the erythrocyte stages of chloroquine-resistant Plasmodium falciparum, having vascular endothelial growth factor inhibitory activity and as medicine for the initial stage of an endothelin receptor agonist. In this study, the yield of asterric acid was improved to a multi-gram quantity from the soft coral-derived fungus Aspergillus sp. through media optimization. The pure spoors of Aspergillus sp. were fermented at room temperature in the absence of sodium chloride salt. The yield of asterric acid was 650 mg/L at optimal conditions. The optimal conditions for the high yield of asterric acid were found as rice medium, room temperature, 56 days incubation and absence of sodium chloride salt.

Keywords: Aspergillus sp., asterric acid, optimal conditions, media optimization, salt concentration, biosynthetic pathway, fermentation, microorganism.

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1. Introduction

Natural products are organic compounds that are produced by organic reaction as a result of an external incentive such as dietary changes, infections and competition. The biological active natural products produced by plants, animals, insects, fungi, bacteria and protozoa are separated for drug design and development (Rollinger *et al.*, 2006; Strohl, 2000). Currently, natural products, herbs, herbal mixtures and nutritional supplements are usually associated with traditional Chinese medicines or secondary medicines (Cai *et al.*, 2018; Clark, 1996). Due to their complex diversity in structure, function and biosynthesis have no definite strategy for their classification. However, natural compounds mainly comprise terpenoids and steroids, fatty acid-derived substances and polychlorinated, alkaloids, non-ribosomal polypeptides and enzyme cofactors. Natural products are important and promising sources for drug discovery and are widely recognized in the pharmaceutical industry because of their structural diversity and broad range of pharmacological activities. Thus, natural products proved to be a paramount source recently and in the coming decades for drug discovery (Blunt *et al.*, 2011). Therefore, natural products are considered useful tools for pharmacologists, biologists and top spot drugs for the treatment of hypercholesterolemia, inflammation, microbial infections and elimination of cancer and transplantation of tissues in organs (Carter, 2011; Ojima, 2008).

According to the estimates fungi are the second largest kingdom after kingdom Animalia. Constant measurement recommended that there are over 1.5 million fungi, among them 5% have been identified by taxonomists (Hawksworth, 1991; Hawksworth, 2001). Fungi are rich in biodiversity and play a variety role, such as the most important role in the health and conservation of ecosystems over the ability to break organic wastes. Fungi can also be used as pathogens, predators, parasites, hosts or co-organisms for animals, plants and other microorganisms (Trappe & Luoma, 1992). Fungi also gained the most adverse amplifications since as the first reported fungal pathogen in 1839. Especially they produce mycotoxins that are associated with various human and animal diseases (Dalldorf, 1962). Mycotoxins and the secondary metabolites produced by fungi are also harmful in the degradation of crops and grains. However, fungi may be used in a profitable way in the food preparation process, beverages and enzymes production on industrial scale (Ainsworth & Sussman, 2013). More importantly, it has been known that fungi have the capability to produce novel bioactive secondary metabolites; among them, some have made significant progress in human health and agriculture and social and economically widespread growth impact (Kubicek & Druzhinina, 2007).

Fungi have been recognized to be multifaceted producer of diverse biologically active secondary metabolites, but still numerous fungi remained chemically unexamined. These considerations strongly insisted that ongoing analysis of fungal chemistry comply with the increasing demand for pharmaceutical and agriculturally beneficial new drugs. The most famous fungal metabolites are penicillins and cephalosporins. Penicillin was the first fungal antibiotics discovered and nomenclature by Fleming in 1928. It was isolated from fungus *Penicillium notatum* and hence named as penicillin. Penicillin is still considered as an important antibiotic and was used by the soldiers as healing for wounds in 2nd World War (Daemmrich & Bowden, 2005). Cephalosporin C was discovered by Brotzu in 1948 from fungus *Cephalosporium acremonium* and showed anti-typhoid activity (Orrù & Riva, 2002). Cephalosporin is nowadays recommended to those patients having allergy from the use of penicillin as an antibiotic. Owing to have broad range of activities and superb safeness making

cephalosporin is one of the largest antibacterial agents worldwide. Therefore, secondary metabolites of fungi still remain an unconventional source for searching of novel drugs (Chin *et al.*, 2006; Newman & Cragg, 2007; Siddiqui *et al.*, 2011; Parish *et al.*, 2008; Corrado & Rodrigues, 2004).

In a unique marine environment, there is fierce competition for survival among marine lives. The large number of invertebrates in the ocean, such as sponges and corals, lack effective physical defensive systems and depend mainly on complex chemical defence mechanisms to survive and reproduce in the competition. Corals are soft-bodied sessile marine invertebrates and it is the chemical defence that enables them to escape predators (Blunt *et al.*, 2017). It is recognized that dense and diverse microbial communities harbouring on and in the tissues of corals are the true producers of many chemical defensive materials (Gerwick & Moore, 2012). Secondary metabolites isolated from coral-associated microorganisms are rich in diversity, including polyketides, terpenoids, meroterpenoids, alkaloids, peptides, shikimates and lipids. Up to date, more than 300 compounds have been obtained with diverse bioactivities, including chemical ecological effects in antifouling, inhibit pathogenic bacteria and deter predators and pharmacological values in antibacterial, antifungal, antiviral and anticancer (Hou *et al.*, 2015). There has been a rapid increase of compounds isolated from coral-associated microorganisms and it still has not reached its climax. Coral mainly protects, adheres and attacks prey by producing infections that are resistant to pathogenic microorganisms, preventing other organisms from attaching and even producing toxic secondary metabolites. Therefore, corals are considered to be an important source of novel and bioactive secondary metabolites (Blunt *et al.*, 2008).

In order to reduce the damage caused by chemical research for the survival of coral, the study found that coral parasites with a large number of microorganisms, these co-organisms can also produce a large number of structurally unique bio-active secondary metabolites to protect the coral in the evolutionary process and can survive in the fierce struggle (Penesyan *et al.*, 2010). Although the link between coral and its associated microorganisms, is not fully understood, but the unique metabolic mechanism of co-organisms of marine origin has made it a hot spot for researchers in recent years. Due to the co-attachment of microorganisms from marine sources, the novel active unique secondary metabolites bring hope for the discovery of new bioactive compounds and thus will help in drug discovery and development. The species in marine ecosystems are not only abundant but also physiologically and structurally characterized. These biological special and physiological structures make them able to survive in high-pressure, salt, light and other complex marine environments. Coral reef ecosystems are an important part of marine ecosystems (Kamel & Slattery, 2005; Liu *et al.*, 2014), where corals and soft corals occupy important biological components. Corals are anchovy-growing, soft invertebrates that survive through physical and chemical defence under the pressure of predators, pathogenic microbes, surface decontamination, space and nutrient competition (Ireland *et al.*, 2000; Liu *et al.*, 2004; Paul *et al.*, 2006; Paul *et al.*, 2011). Studies have shown that the surface and inner tissue of corals have abundant microbial species and microbial biomass (Ainsworth *et al.*, 2010; Bourne *et al.*, 2009; Ritchie, 2006; Harel *et al.*, 2008).

Microbes play an important role in the chemical defence of the hosts in the host-guest relationship. The hosts show the true source of the chemical defensive substances and could be its symbiotic microorganisms. With regard to the sustainable use of resources, the study of microorganisms is more promising but there are only a few cultivable biomasses under the

current conditions. Using modern biological and genomic methods, abundant fungi, bacteria and actinomycetes were isolated and identified with cultivable microorganisms. Among them, fungi were involved in 44 genera and 60 bacterial genera, while actinomycetes have 6 genera. Literature review showed that corals (hosts) were mainly studied from the South China Sea, in addition to the Red Sea, the Pacific coast and the Indian Ocean. The appropriate temperature, salt content, light and nutrients in the South China Sea are more conducive to the growth of organisms. As a result of which the abundant biomasses have been collected, this became a major research area (Hou *et al.*, 2015).

Since 1991, when Fenical and Clardy (Tapiolas *et al.*, 1991) reported for the first time the secondary metabolites of coral-derived microorganisms, the researchers gradually developed secondary metabolites of co-epiphytic microorganisms. By the end of 2016, more than 300 new metabolites were obtained from coral microbes, mainly from fungi, followed by bacteria and actinomycetes. The isolated compounds include polyketides, terpenoids, alkaloids, peptides, shikimic acids and lipids. Polyketides account for half of the total isolated compound's structural type and mainly from the fungus *Aspergillus*, *Alternaria*, *Penicillium* and *Xylariaceae* species. Nitrogenous compounds such as alkaloids and peptides accounted for 33% of the total structure type, mainly reported by the fungus *Aspergillus sp.*, and bacteria *Vibrio*. Studies have shown that polyketides and nitrogenous compounds have a role in biological activities and have the potential to develop antibiotics and antitumor drugs (Hou *et al.*, 2015).

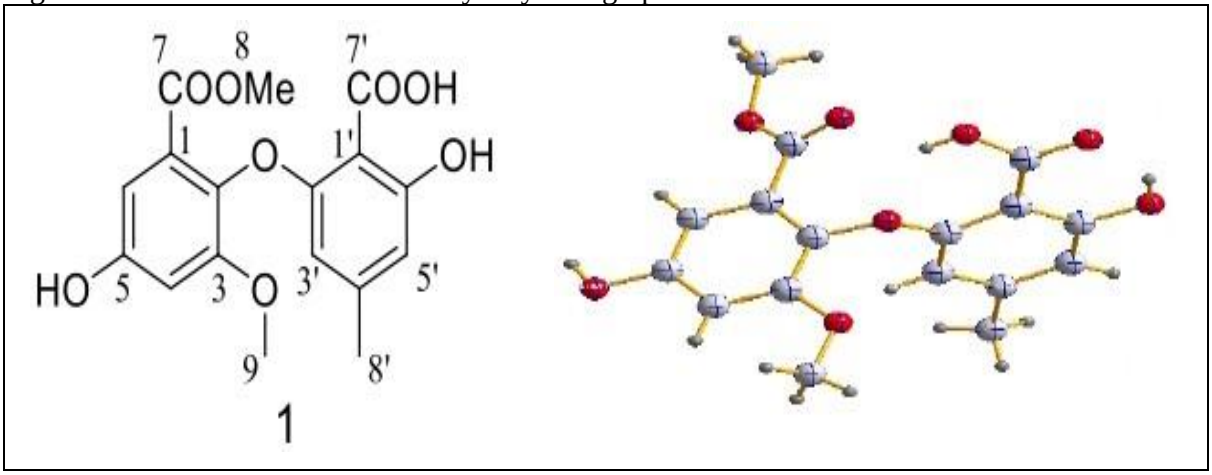
Marine fungi are vital sources of bioactive secondary metabolites (Xing *et al.*, 2016). Metabolites of marine associated fungi have been under investigation for the past few decades which help in the drug discovery for the treatment of different types of diseases (Zhao *et al.*, 2015). Most fungi are cosmopolitan, adopting various habitat like marine environment, soil and freshwater (Bringmann *et al.*, 2007; Lang *et al.*, 2007). Production of bioactive secondary metabolites by microorganisms are cheap and environmentally friendly (Zhao *et al.*, 2016). About 50% of medicines are fungal secondary metabolites, used either in natural or as semi-synthetically modified forms in the pharmaceutical industries (Imhoff, 2016; Demain, 2006). Variation in the fungus culturing condition, the rate of production of metabolites are different from its natural habitat (Nielsen & Smedsgaard, 2003).

Finding a new bioactive fungal secondary metabolite takes a long time and is expensive, however using a suitable culturing condition such as an optimal medium could enhance novel and high production of bioactive metabolites (Boruta & Bizukojc, 2016). Optimization process could lead us to get the secondary metabolites in high yield biosynthetically, improving the formation of desired compounds and minimize or exclude the synthesis of unwanted products (Chomcheon *et al.*, 2009). The high productivity of fungal metabolites could be determined after finding an optimal condition for the growth of a fungus which may have some essential ingredients in minor quantity (Demain, 2006). Among the filamentous fungi *Aspergillus* sp. synthesise a wide range of bioactive secondary metabolites and is therefore considered to be a reservoir for finding novel bioactive natural products (Pang *et al.*, 2017; Wang *et al.*, 2016).

As our ongoing research on fungal bioactive natural products from the soft coral endophytic fungus A*spergillus* sp. we had isolated a minor diphenyl secondary metabolite; asterric acid (figure 1). Asterric acid and its analogues have several interesting bioactivities like antifungal, antimicrobial, cytotoxic, fungicidal, pesticidal, nematicidal, seed germination inhibition (Liu

et al., 2015; Lai *et al.*, 2012; Liu *et al.*, 2006; Li, 2008), anti-HIV activity (Hu *et al.*, 2012), as a first non-peptide for endothelin binding inhibition (Ohashi *et al.*, 1992; Ogawa *et al.*, 1995), anti-plasmodial activity against the erythrocyte stages of chloroquine resistant *Plasmodium falciparum* (Talontsi *et al.*, 2014), α-glucosidase inhibitory activities (Wang *et al.*, 2016; Zhang *et al.*, 2016), having vascular endothelial growth factor inhibitory activity (Lee *et al.*, 2002) and as medicine for the initial stage of endothelin receptor agonist (Clozel *et al.*, 2007). In this study, the yield of asterric acid is improved to multi gram quantity, through media optimization. The yield of asterric acid 1 was 650 mg/L, containing about 60g sterile white rice medium in a 1000ml Erlenmeyer flask.

Figure 1: Chemical formula and X-rays crystallographic structure of Asterric acid 1



2. Materials and methods

2.1. Fungal isolation and identification

The strain (SYM-02-005) was isolated from the inner soft tissue of soft coral *Sinularia* sp. (SYM-02). Based on the RNA nucleotide sequences, it was recognize as *Aspergillus* sp. Its 512 ITS base pair had 98% resemblance to the *Aspergillus* sp. (GenBank No. HQ288052). The fungus has been stored in the Key Laboratory, School of Medicine and Pharmacy, Ocean University of China, Qingdao, Peoples Republic of China, with Genbank accession number KY235298.

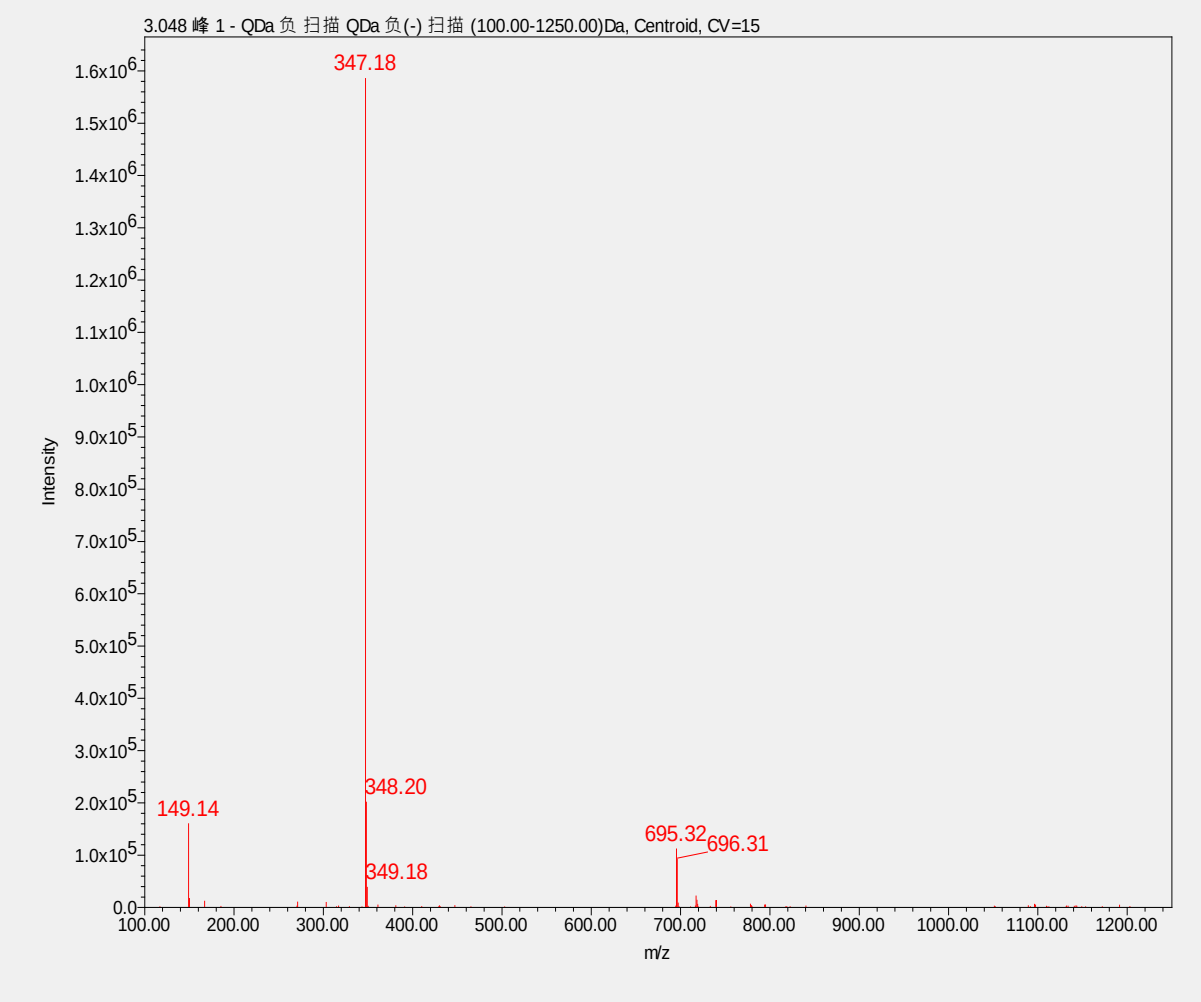
2.2. Fermentation of fungus and extraction of asterric acid 1

The pure spores of strain were streaked on PDA plates and incubated at 28°C for one week, then small plugs of PDA having fungal colonies were aseptically putted into 1000ml Erlenmeyer flasks having about 60g sterilized rice medium as a source of carbon for the fungal growth. The medium was prepared in deionized HPLC grade water and sterilized in autoclave at $121C^0$ and pressure for 20 minutes. The flasks were fermented at room temperature for 7, 14, 21, 28, 56, 84 and 112 days and extracted three times with EtOAc. Each crude extract was concentrated under vacuum and subjected to vacuum liquid chromatography VLC, using silica gel (mesh 200-300), as a stationary phase, eluted with a linear gradient of petroleum ether-EtOAc as a mobile phase. Friction 5 eluted with 50% EtOAc was further subjected to recrystallization using 50:50 MeOH/CH_2Cl_2 as a solvent, asterric acid was obtained as a white solid. The yield of asterric acid was obtained as an average of the triplicate fermented flasks.

2.3. Structural elucidation of asterric acid 1

The ESI-MS spectra (figure 2) were measured on Ultra Performance Liquid Chromatographic Mass Spectrometer, Waters. NMR spectra were recorded on a JEOL JEM-ECP NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR respectively) and TMS as an internal standard while DMSO-d_6 as a solvent.

Figure 2: ESI-MS spectra of Asterric acid 1



3. Results

As a combination of ^{1}H, ^{13}C NMR, ESI-MS, HPLC analysis, comparison with literature data (Liu *et al.*, 2015) and X-rays crystallographic crystal structure (figure 1); the structure of Asterric acid 1 was confirmed. The NMR data of 1 is co-existent with the data from the literature (table-1).

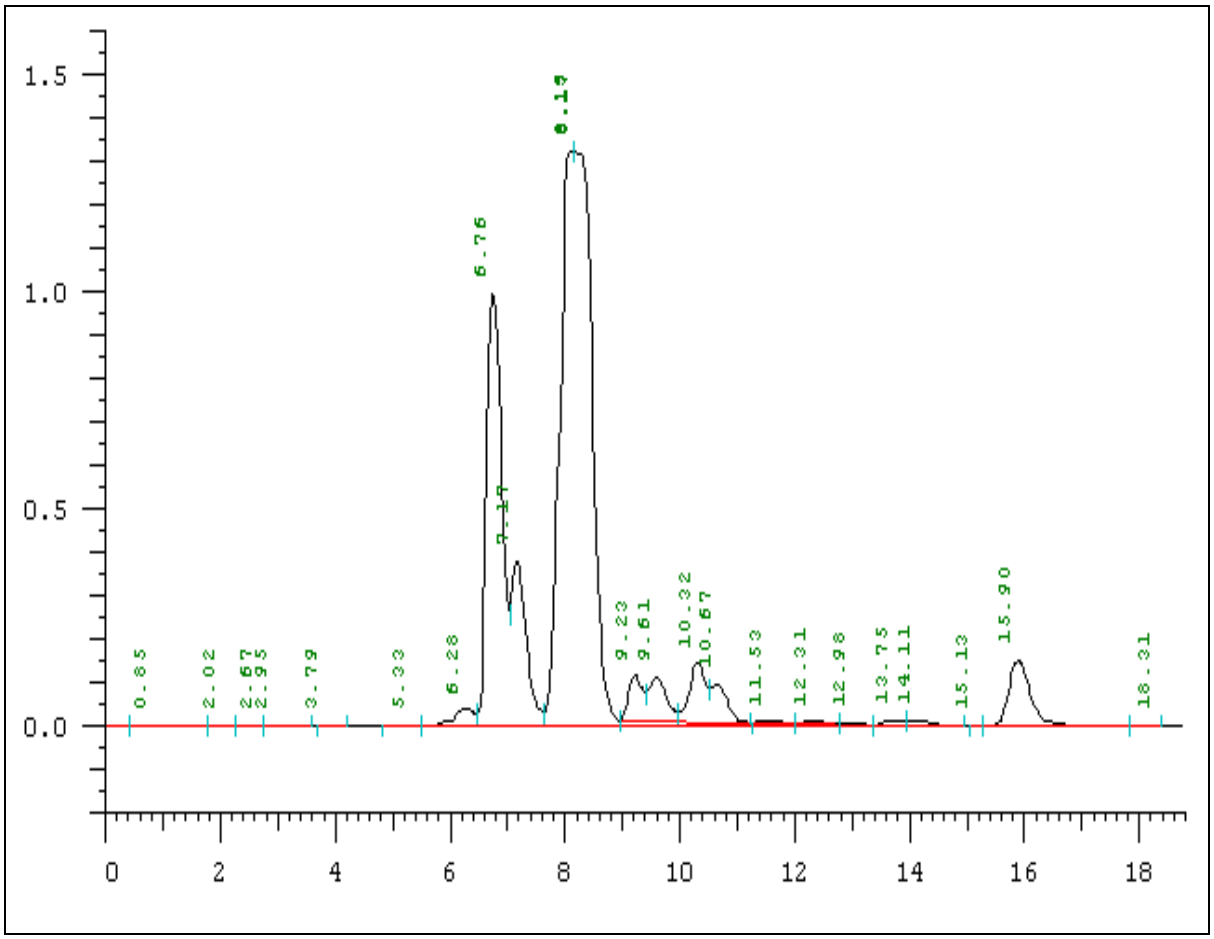
The ESI-MS spectra have ion peaks at *m/z* 347 $[M-H]^-$, suggested the molecular weight of the isolated product is 348 a.m.u (figure 2). According to figure 3, the EtOAc crude extract of asterric acid have a major peak with a retention time 8.1 ($P_{8.1\ min}$). As an average of the pure extract from triplicate flasks, the yield of asterric acid was 650 mg/L after 2, 3 and 4 months of incubation (Figure 4(a)).

Table-1: Comparison of experimental NMR data with literature (Liu *et al.*, 2015) of Asterric acid 1[a]

Position	Data from literature		Data from experiment	
	δ_C	δ_H	δ_C	δ_H
1	125.3		125.3	
2	133.9		133.9	
3	153.4		153.3	
4	104.9	6.78 (1H, s)	105.0	6.77 (1H, s)
5	155.1		155.0	
6	107.5	6.78 (1H, s)	107.5	6.77 (1H, s)
7	165.1		165.0	
8	52.1	3.61 (3H, s)	52.0	3.61(3H, s)
9	56.1	3.70 (3H, s)	56.0	3.70(3H, s)
1'	104.7		104.6	
2'	158.5		158.5	
3'	109.9	6.33 (1H, s)	109.8	6.31 (1H, s)
4'	143.2		143.1	
5'	104.1	5.65 (1H, s)	104.1	5.64 (1H, s)
6'	159.8		159.9	
7'	170.3		170.2	
8'	21.5	2.07 (1H, s)	21.4	2.07 (3H, s)

Measured in DMSO-d_6

Figure 3: HPLC chromatogram of crude extract “A” after 120 days and reference sample “B” of Asterric acid 1

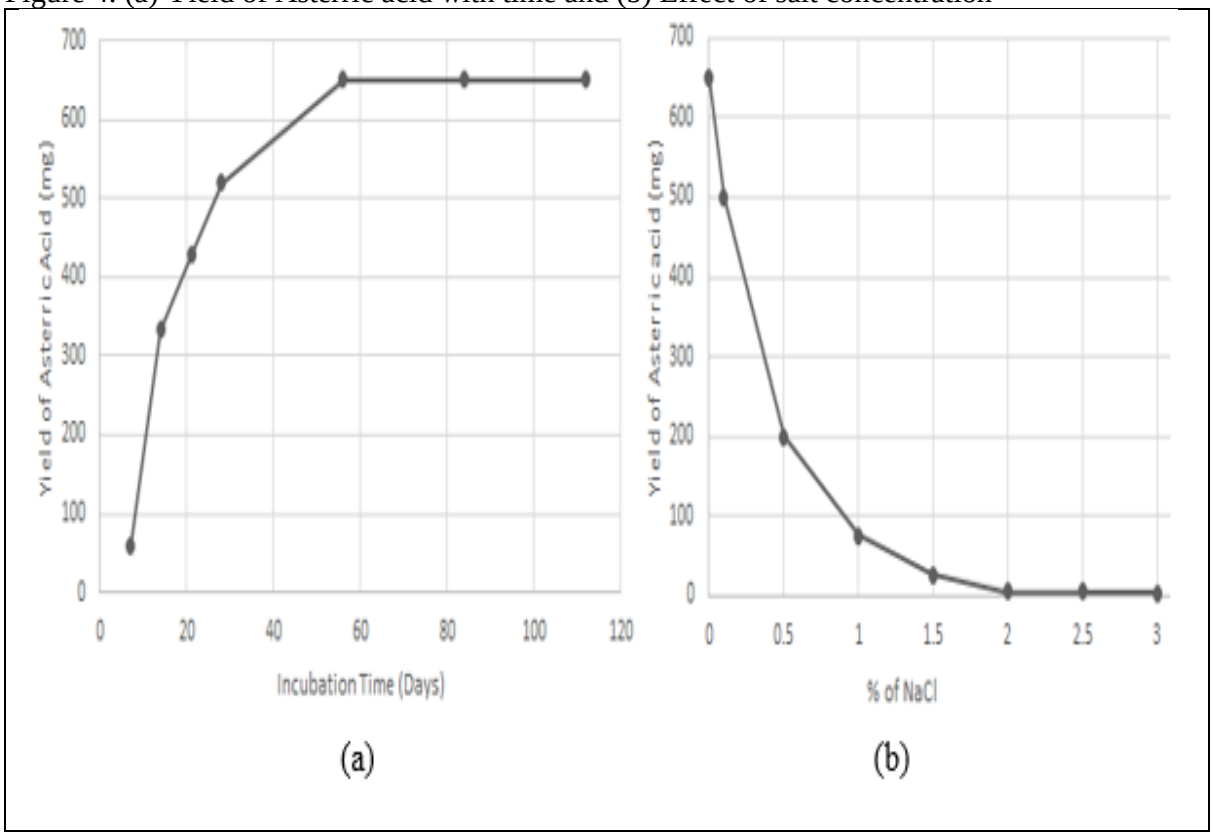


4. Discussion

Asterric acid is a secondary metabolite of several fungal genera (Figueroa *et al.*, 2015). Limited efforts have been made to improve its yield biosynthetically. In 2016 Tomasz Boruta and Marcin Bizukojc got the yield of asterric acid as 2.96 mg/L, using rapeseed oil and excluding sodium chloride in optimal medium during biosynthesis in a stirred tank bioreactor from *Aspergillus terrus* (Boruta & Bizukoic, 2016). Excitingly it is for the first time to improve the yield of asterric acid to multi gram quantity through media optimization from the soft coral derived fungus *Aspergillus* sp. The optimal incubation period for the high yield of asterric acid was 56 days (figure 4(a)); and the best time of fungal cultivation was 112 days, after that the target compound could be easily purified by re-crystallization of the crude extract without using chromatography.

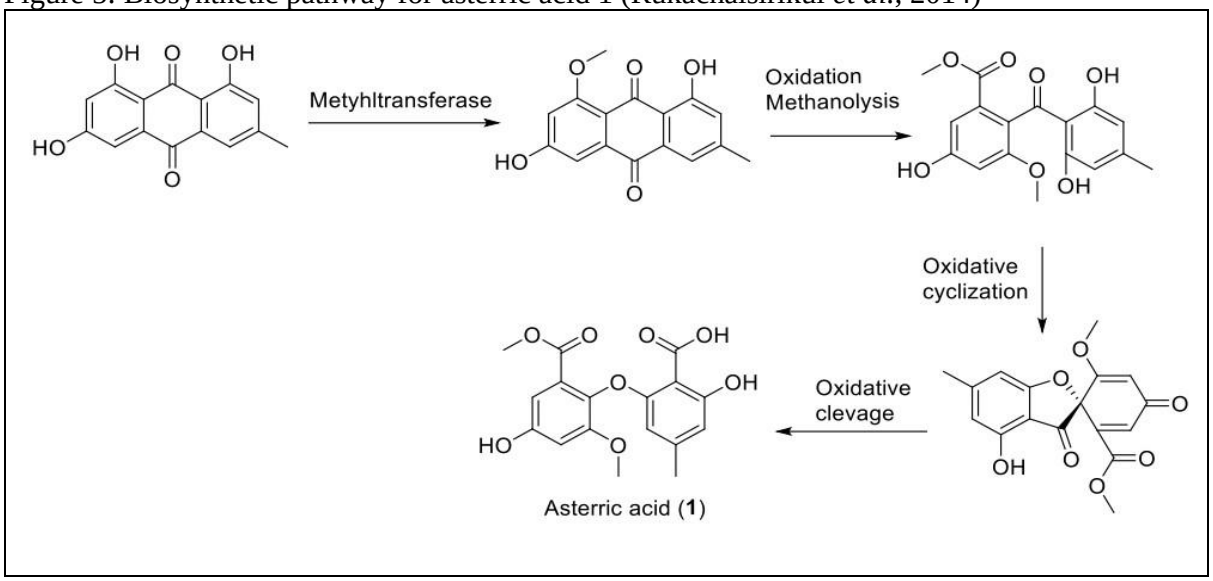
Moreover, in the presence of sodium chloride salt in the medium the production of asterric acid was very low (Boruta & Bizukojc, 2016; Said *et al.*, 2019; Said & Ahmad, 2022). The yield of asterric acid decreases with an increase in salt concentration (figure 4(b)).

Figure 4: (a) Yield of Asterric acid with time and (b) Effect of salt concentration



Several media were used for the optimization processes such as Potato Dextrose Agar (PDA), Starch, Chashi and Glucose Peptone Yeast (GPY) with and without sodium chloride salt. However, the optimal conditions for the high yield of asterric acid was found as rice medium without sodium chloride salt and 56 days incubation period. Furthermore, the hypothetically proposed biosynthetic pathway (figure 5) for asterric acid 1 was also confirmed (Said *et al.*, 2019; Said *et al.*, 2018; Rukachaisirikul *et al.*, 2014).

Figure 5: Biosynthetic pathway for asterric acid 1 (Rukachaisirikul *et al.*, 2014)



5. Conclusions

In presence of sodium chloride salt the yield of asterric acid was very low and the biosynthetic pathway was greatly affected by salt concentration. In absence of sodium chloride salt *Aspergillus* sp was found to produce non-chlorinated secondary metabolites and asterric acid was the major product while in its presence it produced Geodin a chlorinated major product (Said & Ahmed, 2022). The process is simple, inexpensive, and environmentally friendly and the target compound could be obtained via recrystallization of the crude extract without using any chromatography. It is further concluded that dechlorinated secondary metabolites produced by this species such as asterric acid are the sole secondary metabolites while those having chlorine atoms in their structure are due to the presence of chlorinating agents in the fermentation medium, such as sodium chloride salt.

Declaration of conflict of interest

The author(s) declared no potential conflicts of interest(s) with respect to the research, authorship, and/or publication of this article.

Ethics declarations

This article does not contain any studies involving human participants or animals performed by any of the authors.

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References

Ainsworth, G. C., & Sussman, A. S. (Eds.). (2013). *The fungal population: an advanced treatise*. Elsevier.

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends in Ecology & Evolution*, *25*(4), 233-240. https://doi.org/10.1016/j.tree.2009.11.001

Blunt, J. W., Copp, B. R., Hu, W. P., Munro, M. H., Northcote, P. T., & Prinsep, M. R. (2008). Marine natural products. *Natural Product Reports*, *25*(1), 35-94. https://doi.org/10.1039/B701534H

Blunt, J. W., Copp, B. R., Munro, M. H., Northcote, P. T., & Prinsep, M. R. (2011). Marine natural products. *Natural Product Reports*, *28*(2), 196-268. https://doi.org/10.1039/C005001F

Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H., & Prinsep, M. R. (2017). Marine natural products. *Natural Product Reports*, *34*(3), 235-294. https://doi.org/10.1039/C6NP00124F

Boruta, T., & Bizukojc, M. (2016). Induction of secondary metabolism of Aspergillus terreus ATCC 20542 in the batch bioreactor cultures. *Applied Microbiology and Biotechnology*, *100*(7), 3009-3022. https://link.springer.com/article/10.1007/s00253-015-7157-1

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. D. (2009). Microbial disease and the coral holobiont. *Trends in Microbiology*, *17*(12), 554-562. https://doi.org/10.1016/j.tim.2009.09.004

Bringmann, G., Gulder, T. A., Lang, G., Schmitt, S., Stöhr, R., Wiese, J., ... & Imhoff, J. F. (2007). Large-scale biotechnological production of the antileukemic marine natural product sorbicillactone A. *Marine Drugs*, *5*(2), 23-30. https://doi.org/10.3390/md502023

Cai, T., Guo, Z. Q., Xu, X. Y., & Wu, Z. J. (2018). Recent (2000–2015) developments in the analysis of minor unknown natural products based on characteristic fragment information using LC–MS. *Mass Spectrometry Reviews*, *37*(2), 202-216. https://doi.org/10.1002/mas.21514

Carter, G. T. (2011). Natural products and Pharma 2011: Strategic changes spur new opportunities. *Natural Product Reports*, *28*(11), 1783-1789. https://doi.org/10.1039/C1NP00033K

Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *The AAPS Journal*, *8*(2), E239-E253. https://link.springer.com/article/10.1007/BF02854894

Chomcheon, P., Wiyakrutta, S., Sriubolmas, N., Ngamrojanavanich, N., Mahidol, C., Ruchirawat, S., & Kittakoop, P. (2009). Metabolites from the endophytic mitosporic Dothideomycete sp. LRUB20. *Phytochemistry*, *70*(1), 121-127. https://doi.org/10.1016/j.phytochem.2008.10.007

Clark, A. M. (1996). Natural products as a resource for new drugs. *Pharmaceutical Research*, *13*(8), 1133-1141. https://link.springer.com/article/10.1023/A:1016091631721

Clozel, M., Gatfield, J., & Roux, S. (2007*). Endothelin receptor antagonists for early stage idiopathic pulmonary fibrosis*. WO Patent 2007119214.

Corrado, M., & Rodrigues, K. F. (2004). Antimicrobial evaluation of fungal extracts produced by endophytic strains of Phomopsis sp. *Journal of Basic Microbiology: An*

International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms, *44*(2), 157-160. https://doi.org/10.1002/jobm.200310341

Daemmrich, A., & Bowden, M. E. (2005). Top pharmaceuticals: a look at drugs that changed our world. *Chemical & Engineering News*, *83*(25), 3-5.

Demain, A. L. (2006). From natural products discovery to commercialization: a success story. *Journal of Industrial Microbiology and Biotechnology*, *33*(7), 486-495. https://doi.org/10.1007/s10295-005-0076-x

Figueroa, L., Jiménez, C., Rodríguez, J., Areche, C., Chávez, R., Henríquez, M., ... & Vaca, I. (2015). 3-Nitroasterric acid derivatives from an Antarctic sponge-derived Pseudogymnoascus sp. fungus. *Journal of Natural Products*, *78*(4), 919-923.

Gerwick, W. H., & Moore, B. S. (2012). Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. *Chemistry & Biology*, *19*(1), 85-98. https://doi.org/10.1016/j.chembiol.2011.12.014

Harel, M., Ben-Dov, E., Rasoulouniriana, D., Siboni, N., Kramarsky-Winter, E., Loya, Y., ... & Kushmaro, A. (2008). A new Thraustochytrid, strain Fng1, isolated from the surface mucus of the hermatypic coral Fungia granulosa. *FEMS Microbiology Ecology*, *64*(3), 378-387. javascript: https://doi.org/10.1111/j.1574-6941.2008.00464.x

Hou, X. M., Xu, R. F., Gu, Y. C., Wang, C. Y., & Shao, C. L. (2015). Biological and chemical diversity of coral-derived microorganisms. *Current Medicinal Chemistry*, *22*(32), 3707-3762.

Hu, G., Li, X., Li, Y., Sun, X., Liu, G., Li, W., ... & Tang, Y. (2012). Inhibitors of HIV-1 Integrase-Human LEDGF/p75 interaction identified from natural products via virtual screening. *Chinese Journal of Chemistry*, *30*(12), 2752-2758. https://doi.org/10.1002/cjoc.201200897

Hawksworth, D. L. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research*, *95*(6), 641-655. https://doi.org/10.1016/S0953-7562(09)80810-1

Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1· 5 million species estimate revisited. *Mycological Research*, *105*(12), 1422-1432. https://doi.org/10.1017/S0953756201004725

Imhoff, J. F. (2016). Natural products from marine fungi—Still an underrepresented resource. *Marine Drugs*, *14*(1), 19. https://doi.org/10.3390/md14010019

Kamel, H. N., & Slattery, M. (2005). Terpenoids of Sinularia.: chemistry and biomedical applications. *Pharmaceutical Biology*, *43*(3), 253-269. https://doi.org/10.1080/13880200590928852

Kubicek, C. P., & Druzhinina, I. S. (Eds.). (2007). *Environmental and microbial relationships*. Springer.

Lang, G., Wiese, J., Schmaljohann, R., & Imhoff, J. F. (2007). New pentaenes from the sponge-derived marine fungus Penicillium rugulosum: structure determination and biosynthetic studies. *Tetrahedron*, *63*(48), 11844-11849. https://doi.org/10.1016/j.tet.2007.09.025

Lee, H. J., Lee, J. H., Hwang, B. Y., Kim, H. S., & Lee, J. J. (2002). Fungal metabolites, asterric acid derivatives inhibit vascular endothelial growth factor (VEGF)-induced tube formation of HUVECs. *The Journal of Antibiotics*, *55*(6), 552-556. https://doi.org/10.7164/antibiotics.55.552

Li, Y., Sun, B., Liu, S., Jiang, L., Liu, X., Zhang, H., & Che, Y. (2008). Bioactive asterric acid derivatives from the Antarctic ascomycete fungus Geomyces sp. *Journal of Natural Products*, *71*(9), 1643-1646.

Liu, D., Yan, L., Ma, L., Huang, Y., Pan, X., Liu, W., & Lv, Z. (2015). Diphenyl derivatives from coastal saline soil fungus Aspergillus iizukae. *Archives of Pharmacal Research*, *38*(6), 1038-1043. https://link.springer.com/article/10.1007/s12272-014-0371-z

Liu, N., Li, Y. X., Li, Z. Y., Zhong, M., & Lei, X. L. (2014). Advances in the natural products from coral-derived fungi and their activities. *Chin. J. Mar. Drugs*, *33*, 73-84.

Liu, R., Zhu, W., Zhang, Y., Zhu, T., Liu, H., Fang, Y., & Gu, Q. (2006). A new diphenyl ether from marine-derived fungus Aspergillus sp. BF-2. *The Journal of Antibiotics*, *59*(6), 362-365. https://www.nature.com/articles/ja200652

Li, Y., Sun, B., Liu, S., Jiang, L., Liu, X., Zhang, H., & Che, Y. (2008). Bioactive asterric acid derivatives from the Antarctic ascomycete fungus Geomyces sp. *Journal of Natural Products*, *71*(9), 1643-1646.

Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, *70*(3), 461-477.

Nielsen, K. F., & Smedsgaard, J. (2003). Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography–UV–mass spectrometry methodology. *Journal of Chromatography A*, *1002*(1-2), 111-136. https://doi.org/10.1016/S0021-9673(03)00490-4

Ohashi, H., Akiyama, H., Nishikori, K., & Mochizuki, J. I. (1992). Asterric acid, a new endothelin binding inhibitor. *The Journal of Antibiotics*, *45*(10), 1684-1685.

Ogawa, T., Ando, K., Aotani, Y., Shinoda, K., Tanaka, T., TSUKUDA, E., ... & Matsuda, Y. (1995). RES-1214-1 and-2, novel non-peptidic endothelin type A receptor antagonists produced by Pestalotiopsis sp. *The Journal of antibiotics*, *48*(12), 1401-1406. https://doi.org/10.7164/antibiotics.48.1401

Ojima, I. (2008). Modern natural products chemistry and drug discovery. *Journal of Medicinal*, *51*(9), 2587-2588. https://doi.org/10.1021/jm701291u

Orrù, B., & Riva, A. (2002, September). Giuseppe Brotzu and the Discovery of Cephalosporins. In *8th Eurorean Conference of Medical and Health Libraries, Cologne*.

Pang, X., Zhao, J. Y., Fang, X. M., Zhang, T., Zhang, D. W., Liu, H. Y., ... & Yu, L. Y. (2017). Metabolites from the plant endophytic fungus Aspergillus sp. CPCC 400735 and their anti-HIV activities. *Journal of Natural Products*, *80*(10), 2595-2601. https://doi.org/10.1021/acs.jnatprod.6b00878

Paul, V. J., Puglisi, M. P., & Ritson-Williams, R. (2006). Marine chemical ecology. *Natural Product Reports*, *23*(2), 153-180. https://doi.org/10.1039/B404735B

Paul, V. J., Ritson-Williams, R., & Sharp, K. (2011). Marine chemical ecology in benthic environments. *Natural Product Reports*, *28*(2), 345-387.

Parish, C. A., Smith, S. K., Calati, K., Zink, D., Wilson, K., Roemer, T., ... & Harris, G. (2008). Isolation and structure elucidation of parnafungins, antifungal natural products that inhibit mRNA polyadenylation. *Journal of the American Chemical Society*, *130*(22), 7060-7066.

Penesyan, A., Kjelleberg, S., & Egan, S. (2010). Development of novel drugs from marine surface associated microorganisms. *Marine Drugs*, *8*(3), 438-459. https://www.mdpi.com/1660-3397/8/3/438

Ritchie, K. B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, *322*, 1-14. https://www.int-res.com/abstracts/meps/v322/p1-14/

Rollinger, J. M., Langer, T., & Stuppner, H. (2006). Strategies for efficient lead structure discovery from natural products. *Current Medicinal Chemistry*, *13*(13), 1491-1507. https://doi.org/10.2174/092986706777442075

Rukachaisirikul, V., Satpradit, S., Klaiklay, S., Phongpaichit, S., Borwornwiriyapan, K., & Sakayaroj, J. (2014). Polyketide anthraquinone, diphenyl ether, and xanthone derivatives from the soil fungus Penicillium sp. PSU-RSPG99. *Tetrahedron*, *70*(34), 5148-5152. https://doi.org/10.1016/j.tet.2014.05.105

Said, G., Hou, X. M., Liu, X., Chao, R., Jiang, Y. Y., Zheng, J. Y., & Shao, C. L. (2019). Antimicrobial and cytotoxic activities of secondary metabolites from the soft coral derived fungus Aspergillus sp. *Chemistry of Natural Compounds*, *55*(3), 531-533. https://link.springer.com/article/10.1007/s10600-019-02732-5

Said, G., Mou, X. F., Fang, Y. W., Liang, T. M., Wei, M. Y., Chen, G. Y., & Shao, C. L. (2018). Secondary metabolites isolated from the soft coral-derived fungus Aspergillus sp. from the South China Sea. *Chemistry of Natural Compounds*, *54*(3), 547-549.

Said, G., & Ahmad, F. (2022). Effects of salt concentration on the production of cytotoxic geodin from marine-derived fungus Aspergillus sp. *Turkish Journal of Biochemistry*, *47*(4), 399-402. https://doi.org/10.1515/tjb-2022-0058

Siddiqui, I. N., Zahoor, A., Hussain, H., Ahmed, I., Ahmad, V. U., Padula, D., ... & Krohn, K. (2011). Diversonol and blennolide derivatives from the endophytic fungus Microdiplodia sp.: absolute configuration of diversonol. *Journal of natural products*, *74*(3), 365-373.

Strohl, W. R. (2000). The role of natural products in a modern drug discovery program. *Drug Discovery Today*, *5*(2), 39-41. https://doi.org/10.1016/s1359-6446(99)01443-9

Talontsi, F. M., Lamshöft, M., Douanla-Meli, C., Kouam, S. F., & Spiteller, M. (2014). Antiplasmodial and cytotoxic dibenzofurans from Preussia sp. harboured in Enantia chlorantha Oliv. *Fitoterapia*, *93*, 233-238.

Tapiolas, D. M., Roman, M., Fenical, W., Stout, T. J., & Clardy, J. (1991). Octalactins A and B: cytotoxic eight-membered-ring lactones from a marine bacterium, Streptomyces sp. *Journal of the American Chemical Society*, *113*(12), 4682-4683. https://pubs.acs.org/doi/abs/10.1021/ja00012a048

Trappe, J. M., & Luoma, D. L. (1992). The ties that bind: fungi in ecosystems. In J. Dighton, J. White, & P. Oudemans (eds), *The fungal community: Its organization and role in the ecosystem* (pp. 17-27). Marcel Dekker.

Wang, C., Guo, L., Hao, J., Wang, L., & Zhu, W. (2016). α-Glucosidase inhibitors from the marine-derived fungus Aspergillus flavipes HN4-13. *Journal of Natural products*, *79*(11), 2977-2981.

Xing, Q., Gan, L. S., Mou, X. F., Wang, W., Wang, C. Y., Wei, M. Y., & Shao, C. L. (2016). Isolation, resolution and biological evaluation of pestalachlorides E and F containing both point and axial chirality. *RSC Advances*, *6*(27), 22653-22658. https://doi.org/10.1039/C6RA00374E

Zhang, L. H., Feng, B. M., Zhao, Y. Q., Sun, Y., Liu, B., Liu, F., ... & Pei, Y. H. (2016). Polyketide butenolide, diphenyl ether, and benzophenone derivatives from the fungus Aspergillus flavipes PJ03-11. *Bioorganic & Medicinal Chemistry Letters*, *26*(2), 346-350. https://doi.org/10.1016/j.bmcl.2015.12.009

Zhao, C., Guo, L., Wang, L., Zhu, G., & Zhu, W. (2016). Improving the yield of (+)-terrein from the salt-tolerant Aspergillus terreus PT06-2. *World Journal of Microbiology and Biotechnology, 32*(5), 1-9. https://link.springer.com/article/10.1007/s11274-016-2029-0

Zhao, D. L., Shao, C. L., Gan, L. S., Wang, M., & Wang, C. Y. (2015). Chromone derivatives from a sponge-derived strain of the fungus Corynespora cassiicola. *Journal of Natural Products*, *78*(2), 286-293.