IPS Journal of Advanced and Applied Biochemistry
IPS J. Adv. Appl. Biochem., 1(1): 1-10 (2025)
DOI: https://doi.org/10.54117/ijaab.v1i1.58

Extraction and Elucidation of Antibiotics from the Mycelia of *Aspergillus niger* Isolated from Poultry Farm against Enteric Bacterial Pathogens

Iheukwumere, I.H.[1], Nwike, M. I.[2], Iheukwumere, C.M.[2], Ike, V.E.[3], Obianom, A.O.[3], Ihenatuoha, U.A.[4], Igboanugo, E.U.[5], Ekesiobi, A.O.[6], Okereke, F.O.[7], Obiefuna, O. H.[1] Nnadozie, C.H.[1], Agbaugo, C.F.[1], Oduoye, O.T.[8] , Nwakoby, N.E.[1], Ilechukwu, C. C.[9], Ochibulu, S. C.[1] and Ejike, C. E. [10]

[1]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.
[2]Department of Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Nigeria.
[3]Department of Biology, University of Agriculture and Environmental Sciences Umuagwo, Imo State.
[4]Department of Environmental Health, Technology, Federal College of Animal Health and Production Technology, NVRI, VOM, Plateau State.
[5]Department of Microbiology, Faculty of Applied and Natural Sciences, Legacy University, Okija, Anambra State, Nigeria
[6]Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State
[7]Department of Biological Sciences (Microbiology), Spiritan University, Nneochi, Abia State.
[8]Department of Biotechnology, National Centre for Genetic Resources and Biotechnology (NACGRAB). Ibadan, Nigeria
[9]Department of Biochemistry, Faculty of Natural Science,Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
[10]Department of Medical Microbiology, Chukwuemeka Odumegwu Ojukwu University, Anambra State.

*Corresponding authors e-mail: ik.iheukwumere@coou.edu.ng

Abstract

The World Health Organization (WHO) has now issued a warning that the world is "running out of antibiotics", escalating fears about global antibiotic resistance reaching new heights. Lately, the emergence of drug-resistant bacteria has posed a critical challenge for the treatment of diseases and a gradual increase in the frequency of nosocomial infections. Hence battle for newer antibiotics for bacterial infections became necessary and a major research interest. This study focused on the extraction and elucidation of antibiotics from the mycelia of *Aspergillus niger* isolated from poultry farm soil samples from local poultry farms were randomly collected and screened for the presence of *Aspergillus niger* using standard, microbiological techniques. The isolates were grown in a submerged culture and screened for antibiotics. The inhibitory fractions were precipitated, eluted and purified and elucidated using solvent extraction, Column, thin layer and gas chromatographic techniques, coupled with mass spectrophotometric (GC-MS). The study revealed the presence of *Aspergillus niger* strain CBS513.88 (ANC5), *Aspergillus niger* strain HGH48 (ANH48) and *Aspergillus niger* strain HUS1 (ANH1). The isolates exhibited significant ($P<0.05$) production of potent antibiotics when the pH, temperature, carbon and nitrogen sources were 7.0, 25℃, extracted sugar from *Phoenix dactylifera* (date) fruits and NODZ (prepared from a mixture of *Rhizobium Leguminosarum*, soybean meal and *Arachis* hypogoea nodule meal) respectively. The fractions; E-15-heptadecenal (C1) > 14-pentadecenoic acid (C2) eluted from ANC5 showed significant ($P<0.05$) inhibitory activities against enteric bacterial pathogens; *Escherichia coli* strain HH35 (ECH3), *Salmonella enterica* serovar Typhi strain R27 (SETR7) and *Shigella dysenteriae* strain NCTC9718 (SDN9). Similarly, the fractions; oleic acid (H1) > 1-docosene (H2) > 9-octadecenoic acid (Z), methyl ester (H5) > 1-octadecene (H3) from ANH48 and oleic acid (N1) > 1-docosene (N2) > 1-octadecane (N3) and 2(3H)-furanone dihydro-4-hydroxy (N4) from ANH1, showed significant ($P>0.05$) inhibitory activities against the enteric bacterial pathogens. From the study, ANC5, ANH48 and ANH1 isolated from poultry farms produced significant antibiotics against enteric bacterial pathogens and could be used as alternative antibiotics for nosocomial infections.

Keywords: *Antibiotic resistance, Aspergillus niger, enteric pathogens, GC-MS analysis, poultry farm soil.*

Article History
Received: 05 May 2025
Accepted: 26 May 2025
Published: 31 May 2025

Scan QR code to view•

How to cite this paper: Iheukwumere, I. H. et al. (2025). Extraction and Elucidation of Antibiotics from the Mycelia of Aspergillus niger Isolated from Poultry Farm against Enteric Bacterial Pathogens. *IPS Journal of Advanced and Applied Biochemistry*, *1*(1), 1–10. https://doi.org/10.54117/ijaab.v1i1.58.

Introduction

The name *Aspergillus niger* emanated from the Latin word 'Aspergillum' which means 'holy cup sprinkler', because its shape resembles a sprinkler when viewed under a light microscope (Ano *et al*., 2017). It is a filamentous fungus that appears like a small plant. *A. niger* is frequently called a black mold, because it appears black on fruits and vegetables. It is the most common mold found in the environment and in the soil. The fungus is capable of producing citric acid in large quantity when cultured in a medium that contains sugar (Akinfala *et al*., 2020).

A. niger causes a mild opportunistic respiratory infection in immunocompromised individuals and food spoilage also had been reported (Blin *et al*., 2019). The fungus is capable of thriving in an environment where there is a high or low temperature which could not favour the proliferation of other fungi. *A. niger* is mostly found in habitats where decaying materials are found or in grain storage store due to its saprophytic nature (Cairns *et al*., 2018).

It had been revealed that *A. niger* inhabits soil environments, especially in poultry farm. This could be attributed to its nutrition and further supports its saprophytic mode of feeding as documented by Elissawy *et al*. (2017). In poultry farm, birds droppings and feeds saturate the soil, and these substances are decomposed by *A. niger* releasing energy and carbon required for metabolism.

Also, researchers had shown that *A. niger* is capable of producing antibiotics as its secondary metabolites during the stationary phase of growth (Akinfala *et al*., 2020; Fang *et al*., 2016; Cairns *et al.*, 2018). These antibiotics help the organism to compete favorably in its environment, especially when nutrient availability is limited but are not essential in the proliferation of the fungus. Meanwhile, the metabolism of the organism is not affected by the antibiotics, because of variation in structure with other microorganisms especially bacteria. These antibiotics produced by *A. niger* in its habitats can be optimized when the organism is grown on a suitable medium. Cultivation or growing of *A. niger* can be actualized by providing a suitable medium that mimics or contains nutrients that support the growth of the fungus in its natural environment (Ano *et al*., 2017). For instance, an artificial medium that provides an adequate nutrient can be used such as Potato dextrose broth, Sabouraud dextrose broth, yeast extract agar and broth. The organism proliferates and yields secondary metabolites (antibiotics) which can be extracted using an extraction technique, and also their potency can be elucidated using agar well diffusion as documented by Akinfala *et al*. (2020).

Several researchers had worked on evaluation of secondary metabolites from *Aspergillus* species such as Akinfala *et al*. (2020), Elissawy *et al*. (2019) and Blin *et al*. (2019) but little work had been documented on the extraction and elucidation of antibiotics from the mycelia of *A. niger* isolated from poultry farm. Hence, this study is aimed at extracting and elucidating of antibiotics from the mycelia of *A. niger* isolated from poultry farm. The result obtained from this study would contribute immensely in disease prevention and control.

Materials and Methods

Collection of samples: A total of 300 soil samples from hospital waste dumping site were randomly collected from different sites in Ihiala L.G.A, Anambra State. This was carried out using the method described in the study published by Iheukwumere *et al*. (2021). The litter from the soil surfaces was carefully scrapped out using sterile stainless spoon. The soil auger was derived to a plough depth of 15 cm in the farm land, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thorough mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Isolation of the Fungal Isolates: The media used for this isolation was Sabouraud dextrose agar (SDA/BIOTECH). One gram of the soil sample was weighed into boiling test tube, 5 mL of normal saline was added and shake thoroughly and then make up to 10 mL using the normal saline (10^{-1}dilution). One milliliter of the suspension was added to four milliliter (4 mL) of normal saline (0.85% NaCl), which was give 5^{-1} dilution. From 5^{-1} dilution test tube, a five-fold serial dilution was carried out to obtain 5^{-5} dilution. One milliliter aliquot from 10^{-1}, 5^{-1} and 5^{-5} test tubes were collected and aseptically plated onto solidified sabouraud dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010) supplemented with chloramphenicol (0.05 %) and spread using a spreading rod. The SDA was incubated in an inverted position for 5-7 days at 30±2^{0}C (Iheukwumer *et al*., 2025c).

Identification of Fungal Isolates: The fungal isolates were identified to the genus/species level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures as described in the study published by Iheukwumere *et al*. (2020a) and Iheukwumere *et al*., (2022b).

Screening the Fungal Isolates for Antibiotic production: For antibiotic production, Mueller Hinton Agar (MHA) medium was prepared according to the manufacturer's direction. This was allowed to cool and then poured in Petri dishes and kept in incubator at 37^{0}C for 24 h to check its sterility. Then the test organisms; *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella species* were grown on broth culture at 37^{0}C for 24 h. After incubation, sterilized swab stick was dipped into the broth cultures and swabbed on MHA plates and allowed for 1 h. Then wells were made on the MHA plates using sterile cork borer. Then the broth culture of the fungal isolates were carefully centrifuged at 6000 rpm for 10 minutes and their supernatants were poured into the wells and incubated at 37^{0}C for 48 h. zones of inhibition were observed after incubation (Iheukwumere *et al*., 2025b)).

Extraction of Antibiotics: The characterized fungal isolates were grown in a Brain heart Infusion broth: 10g/L,peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L,

(NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. This was incubated at room temperature (30±2°C) for 7 days with intermittent manual shaking (Iheukwumere *et al*., 2025).

Extraction and Elution of Antibiotic: The culture medium was centrifuged at 8000 rpm for 15 min. This was filtered using What Man No1 filter paper (110 mm × 110 mm). The supernatant was eluted using a column chromatographic technique using ethyl acetate/hexane/methanol/dichloromethane at ration of 2:2:1:1.

***In vitro* Antibacterial Activities of the Eluate using Agar Well Diffusion Method:** This was carried out by the modified method of Iheukwumere and Umedum (2013). Each labeled plate was uniformly inoculated with the test organism (*Escherichia col*i, *Staphylococcus aureus* and *Salmonella* specie) using spread plate method. A sterile cork borer of 5 mm diameter was used to make the wells on the medium. One tenth millilitre of the eluate was dropped into each labeled wells and then incubated at 35±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation (Iheukwumere *et a*l., 2025d)

Purification and Elucidation of the Antibiotic: The eluate that inhibited the growth of the tested bacteria were subjected to Thin Layer Chromatographic technique using chloroform/methanol (24:1 v/v), chloroform/methanol/water (1:1:1 v/v/v), benzene/acetic acid/water (4:1:5 v/v/v) and acetonitrile/water (92.5/7.5 v/v). The successive bands seen on the plates were crapped off carefully, dissolved in methanol, and centrifuged at 10,000 rpm for 10 min to remove the silica. The supernatant was subjected to structural elucidation using gas chromatography coupled with mass-spectrophotometer (Ranjan and Jadeja, 2017).

Data Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated. Significance of the study was carried out using one way Analysis of Variance (ANOVA) at 95% confidence level. Pair wise comparism was carried out using student "t" test (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2020).

Results

The fungal (M,N and O) exhibited similar macroscopic and microscopic characteristics but differed slightly on the later colour on Sabouraud Dextrose agar (SDA) as shown in tables 1 and 2. The molecular characteristics revealed that isolates M,N and O *Aspergillus niger* strain CBS513.88 (ANC5), *Aspergillus niger* strain HG48 (ANH48) and *Aspergillus niger* strain HUS1 (ANH1).

The antibiotics secreted from the fungal isolates showed significant (P<0.05) inhibition against the enteric bacterial pathogens. The fractions (C1>C2) eluted from ANC5 showed significant (P<0.05) activities against enteric bacteria pathogens *Escherichia coli* strain HH35 (ECH3), *Salmonella enterica* serovar Typhi strain R27 (SETR7) and *Shigella dysenteriae* strain NCTC 9718 (SDN9), of which the activity was most against ECH3 but it was statistically non-significant (P>0.05) when compared to the activities against SETR7 and SDN9. C1 and C2 were E-15-heptadecenal and 14-pentadecenoic acid. The fractions (H1>H2>H4>H5>H3) from ANH48 exhibited similar inhibitory activities with the fractions from ANC5 as shown in figures 1-3. Also similar trends was seen in the fractions (N1>N2>N3>N4) eluted from ANH1 as shown in figure 4.

The GC-MS products of ANC5, ANH1 and ANH48 were presented in Figures 5-7. H1, H2, H3, H4 and H5 were Oleic acid, 1-Docosene, 1-Octadecene, 10-Octadecenoic acid, methyl ester and 9-Octaddecenoic acid (Z), methyl ester respectively. N1, N2, N3 and N4 were Oleic acid, 1-docosene, 1-Octadecane and 2(3H)-furanone dihydro-4-hydroxy- respectively.

Table 1: Macroscopic characteristics of the isolates

Parameter	Isolate M	Isolate N	Isolate O
Growth within 2-3 days on SDA	White	White	White to pale
Later growth from 5 days on SDA	Black with white edge	Black with pale edge	Dark brown
Reverse Colour	Pale	Pale	Pale
Growth Rate	Rapid	Rapid	Rapid
Colony texture	Powdery	Powdery	Powdery
Colour	Black	Carbon black	Dark brown
Fungus	*Aspergillus* species	*Aspergillus* species	*Aspegillus* species

Table 2: Microscopic characteristics of the fungal isolates

Parameter	Isolate M	Isolate N	Isolate O
Shape of vesicle	Globose	Globose	Globose
Metula covering	Entire	Entire	Entire
Shape of conidia	Ellipsoidal	Ellipsoidal	Ellipsoidal
Colour of conidia	Black	Carbon black	Dark brown
Conidia head	Radiate	Radiate	Radiate
Texture of conidia	Finely wrinkled	Finely wrinkled	Finely wrinkled
Nature of hyphae	Septate	Septate	Septate
Colour of conidiosphore	Hyaline	Hyaline	Hyaline
Texture of conidiosphore	Smooth	Smooth	Smooth
Length of conidiosphore	Long	Long	Long
Seriation (sterigmata)	Biseriate	Biseriate	Biseriate
Fungus	*Aspergillus niger*	*Aspergillus niger*	*Aspergillus* niger

Table 3: Molecular characteristics of the isolates

Parameter	Isolate M	Isolate N	Isolate O
Max score	944	701	832
Total score	3779	701	832
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession number	NT166520	KX099668	MF163441
Description	*Aspergillus niger* strain CBS513.88 (ANC5)	*Aspergillus niger* strain HG48(ANH48)	*Aspergillus niger* strain HUS 1 (ANH1)

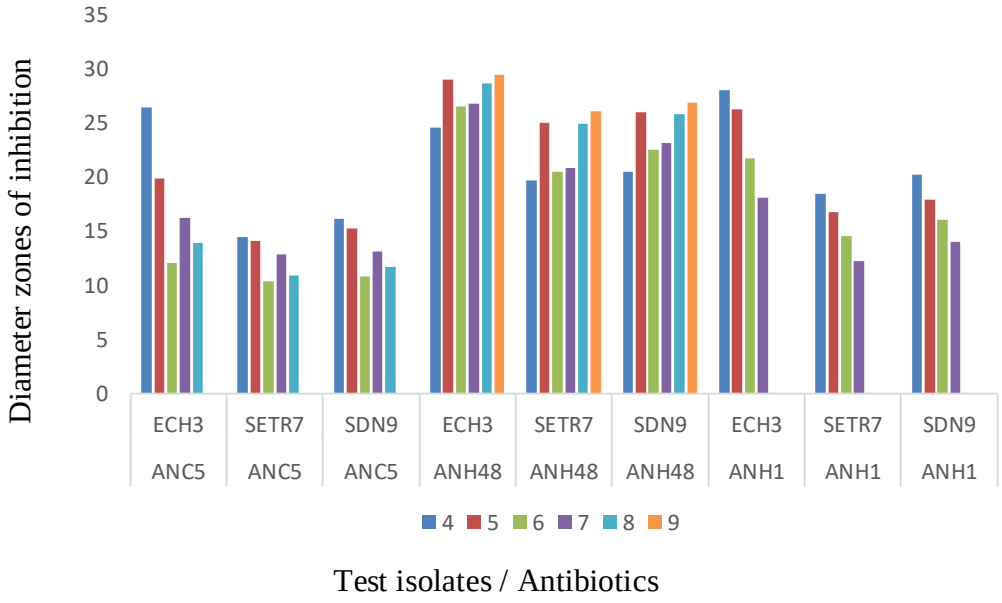

Figure 1: Effects of the production of antibiotics enteric bacterial pathogens
Escherichia coli strain HH35 ---- ECH3, *Salmonella entrica* ser. Typhi strain R27 ----SETR7
Shigella dysenteriae strain NCTC9718 -----SDN9

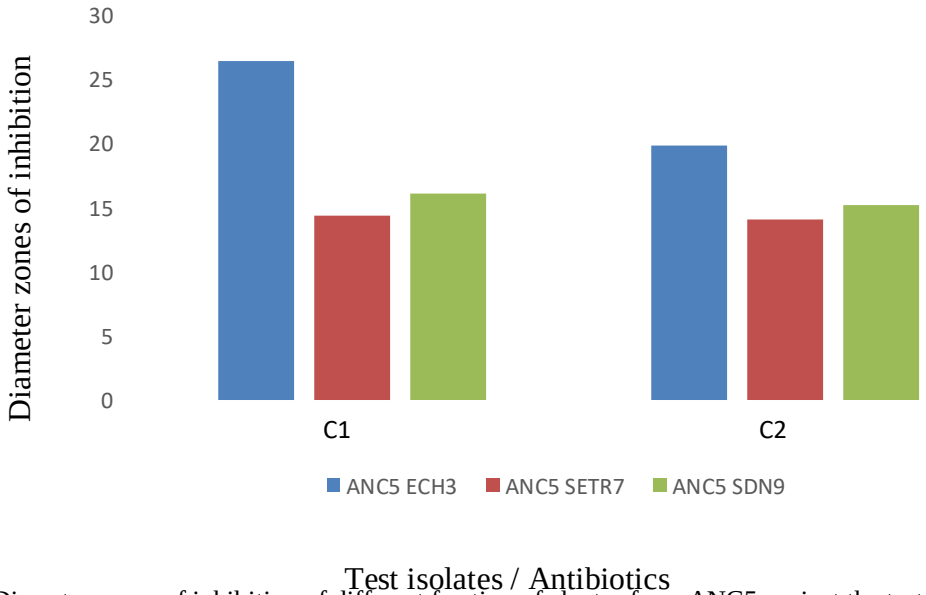

Figure 2: Diameter zones of inhibition of different fraction of eluates from ANC5 against the test isolates.
Escherichia coli strain HH35 -- ECH3, *Salmonella entrica* ser. Typhi strain R27 ---SETR7
Shigella dysenteriae strain NCTC9718 ---- SDN9

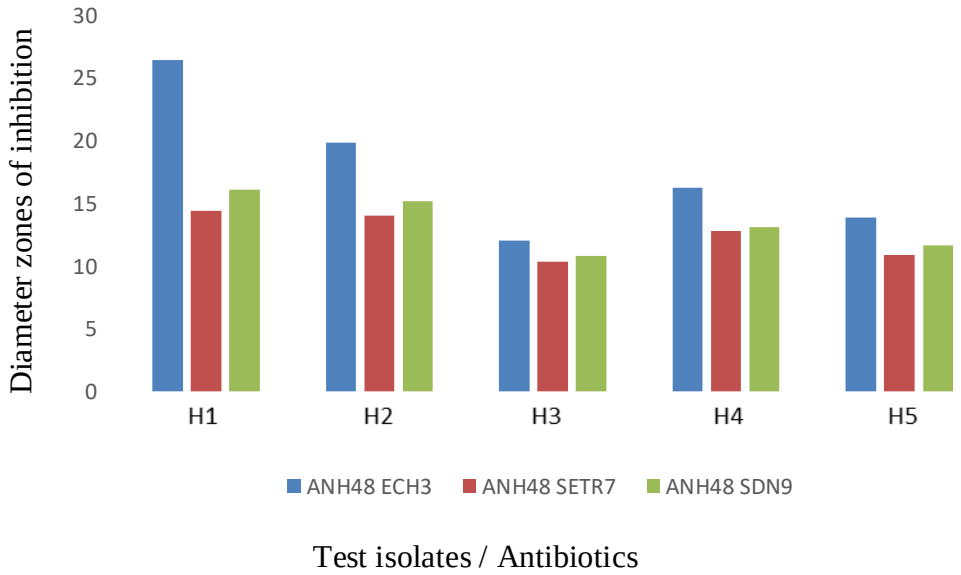

Figure 3: Diameter zones of inhibition of different fraction of eluates from ANH48 against the test isolates.
Escherichia coli strain HH35 ---ECH3, *Salmonella entrica* ser. Typhi strain R27 -- SETR7
Shigella dysenteriae strain NCTC9718 ----SDN9

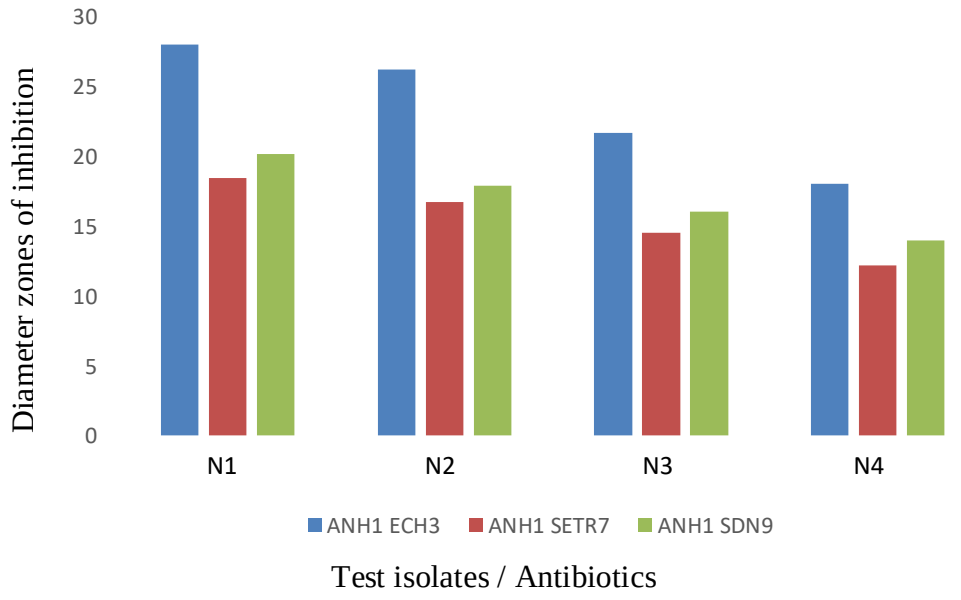

Figure 4: Diameter zones of inhibition of different fraction of eluates from ANH1 against the test isolates.
Escherichia coli strain HH35-- ECH3, *Salmonella entrica* ser. Typhi strain R27 -- SETR7
Shigella dysenteriae strain NCTC9718 -- SDN9

Table 4: GC/MC products from ANC5 eluates

Fraction	Product	Molecular Formula
C1	E-15-Heptadecenal	$C_{17}H_{32}O$
C2	14-Pentadecenoic acid	$C_{15}H_{28}O_2$

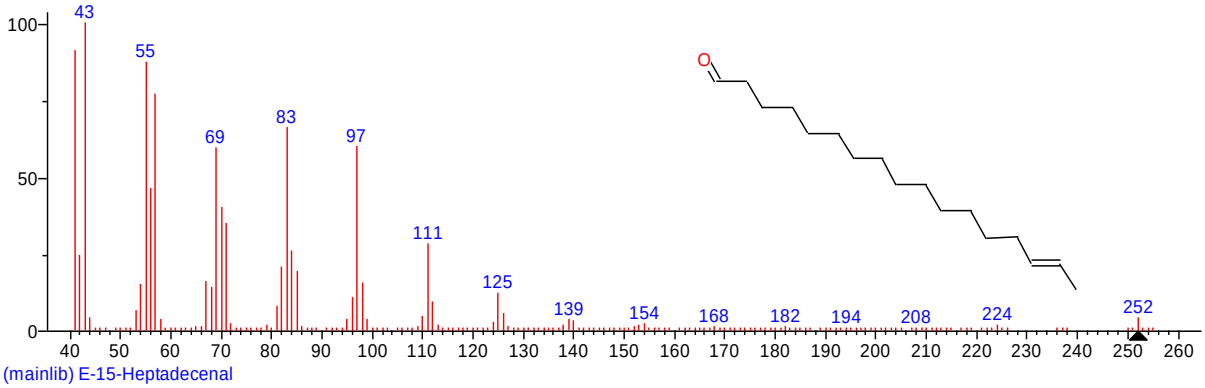

Figure 5a: Mass spectral of ANC5 eluates: E-15-Heptadecenal

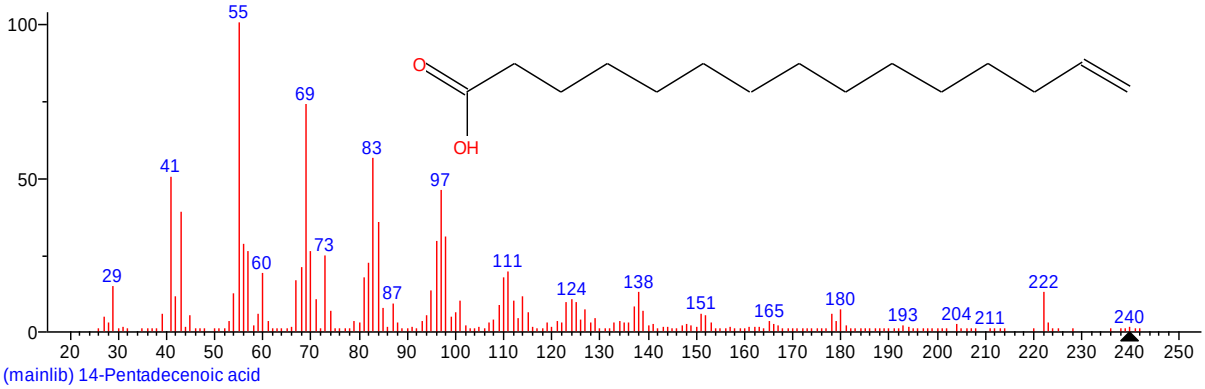

Figure 5b: Mass spectral of ANC5 eluates: 14-Pentadecenoic acid

Table 5: GC/MC products from ANH1 eluates

Fraction	Product	Molecular Formula
N1	Oleic Acid	$C_{18}H_{34}O_2$
N2	1-Docosene	$C_{22}H_{44}$
N3	1-Octadecene	$C_{18}H_{36}$
N4	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$

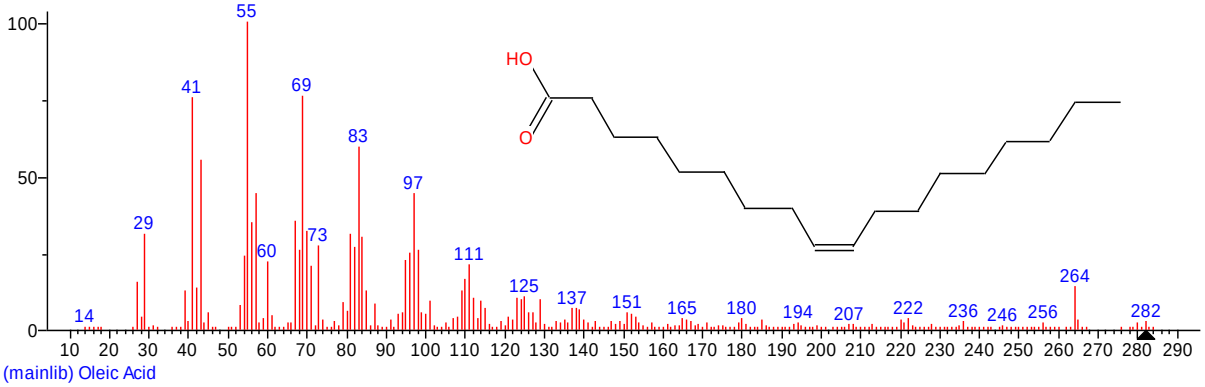

Figure 6a: Mass spectral of ANH1 eluates: Oleic Acid

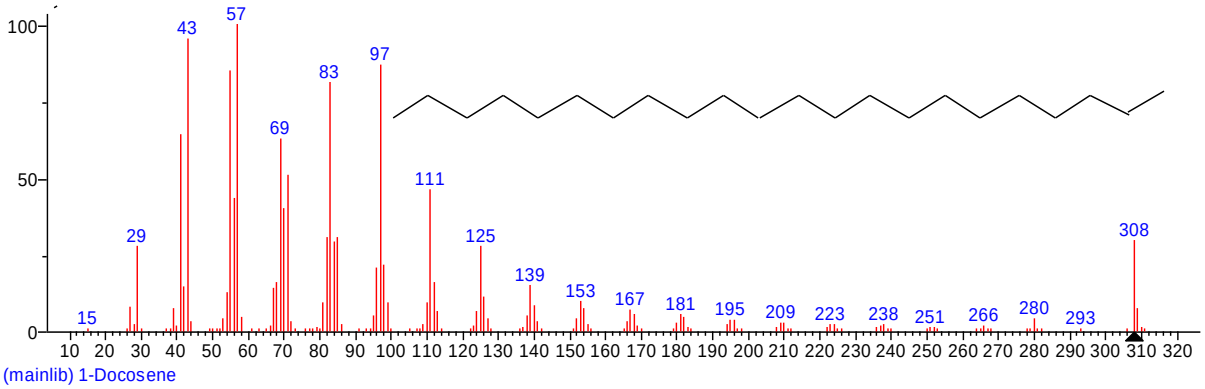

Figure 6b: Mass spectral of ANH1 eluates: 1-Docosene

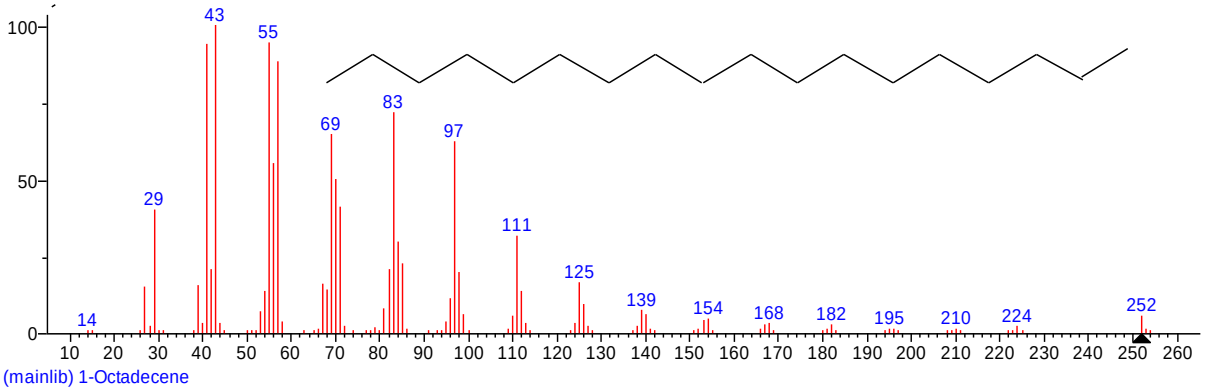

Figure 6c: Mass spectral of ANH1 eluates: 1-Octadecene

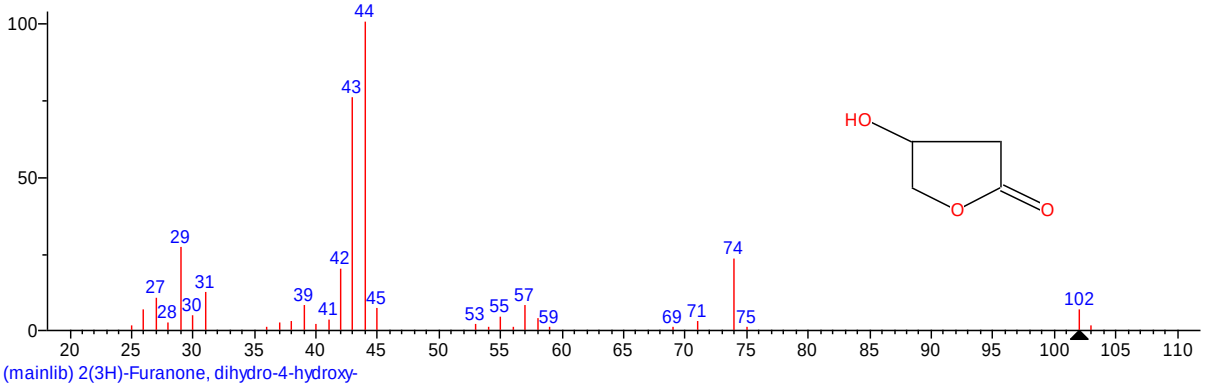

Figure 6d: Mass spectral of ANH1 eluates: 2(3H)-Furanone, dihydro-4-hydroxy-

Table 6: GC/MC products from ANH48 eluates

Fraction	Product	Molecular Formula
H1	Oleic Acid	$C_{18}H_{34}O_2$
H2	1-Docosene	$C_{22}H_{44}$
H3	1-Octadecene	$C_{18}H_{36}$
H4	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
H5	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$

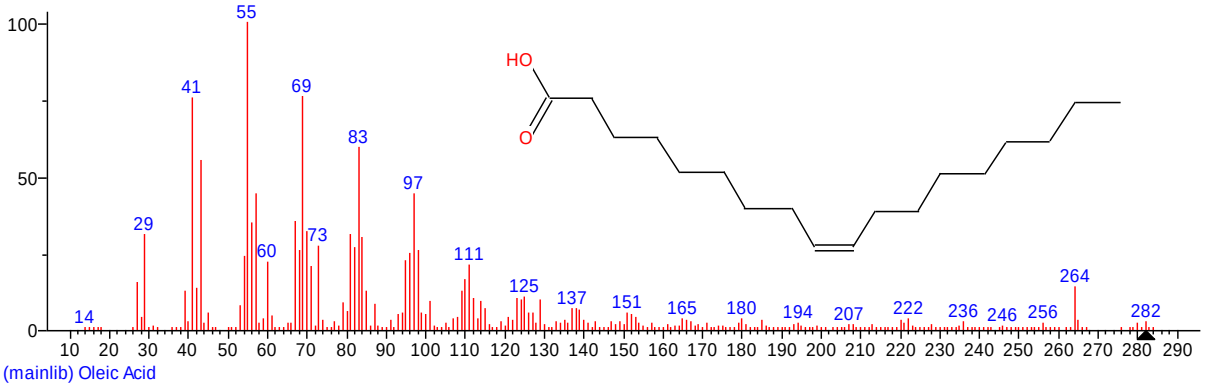

Figure 7a: Mass spectral of ANH48 eluates: Oleic Acid

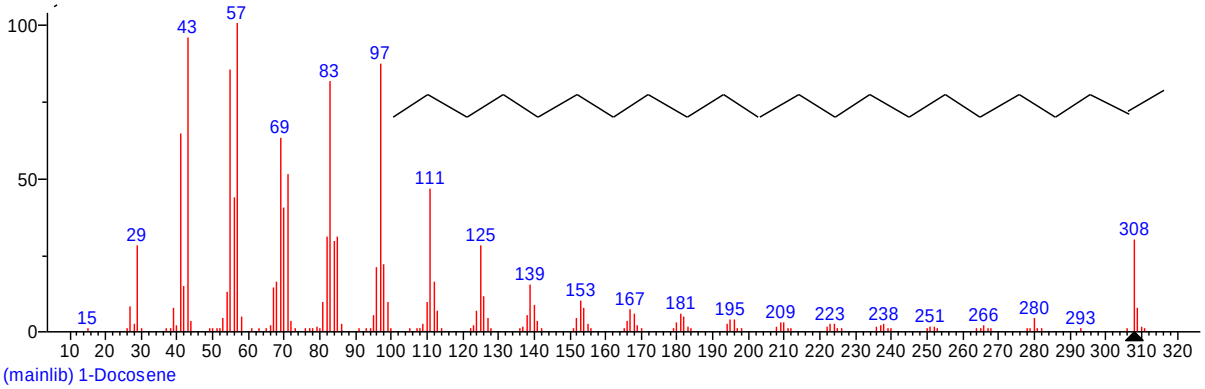

Figure 7b: Mass spectral of ANH48 eluates: 1-Docosene

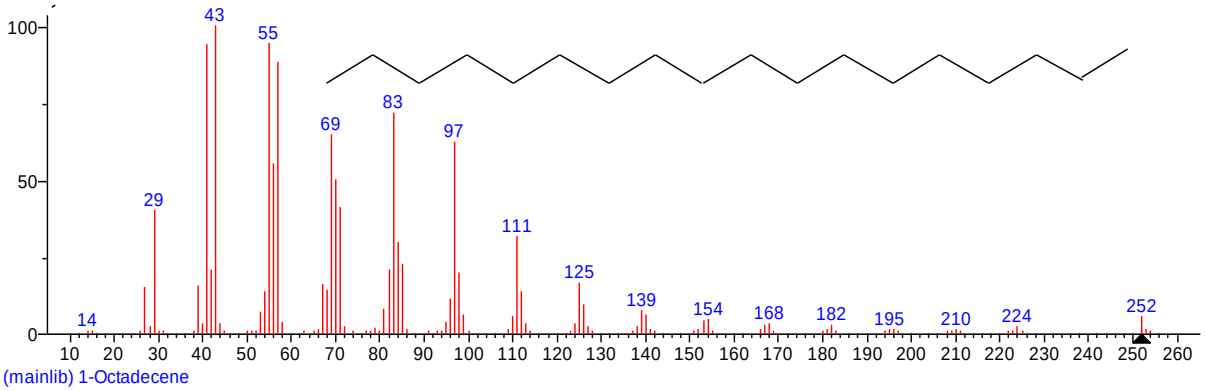

Figure 7c: Mass spectral of ANH48 eluates: 1-Octadecene

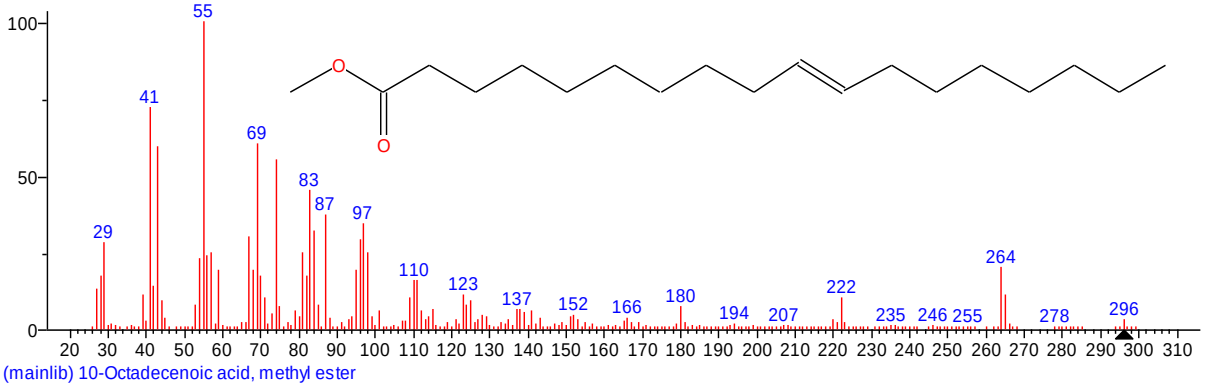

Figure 7d: Mass spectral of ANH48 eluates: 10-Octadecenoic acid, methyl ester

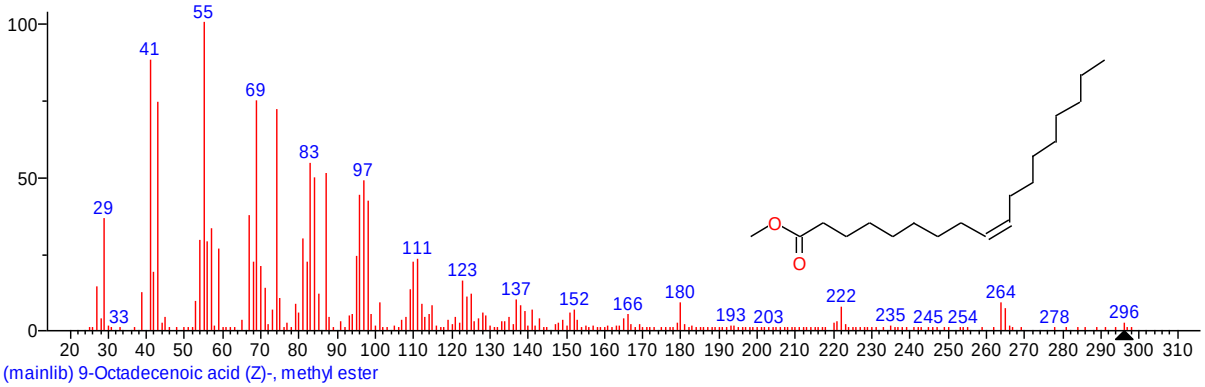

Figure 7e: Mass spectral of ANH48 eluates: 9-Octadecenoic acid (Z)-, methyl ester

Discussion

The characteristic features of *Aspergillus niger* strain CBS513.88 (ANC5), *Aspergillus niger* strain HG48 (ANH48) and *Aspergillus niger* strain HUS1 (ANH1) isolated from poultry farms in the present study supported the reports of many researchers (Astoreca *et al*., 2011; Akhtar *et al*., 2014; Ashraf *et al*., 2015; Habib *et al*., 2015).

The production antibiotics from different strains of *Aspergillus niger* as witnessed in the present study supported the findings of many researchers (Fawzy *et al*., 2011; Abdulwahid *et al*., 2013; An *et al*., 2019, Guo *et al*., 2021; Wei *et al*., 2022) who extracted antibiotics in various form during their studies.

The inhibitory activities of the eluted and purified fractions against enteric bacterial pathogens; *Escherichia coli* strain HH35 (ECH3), *Salmonella enterica* serovar Typhi strain R27 (SETR7) and *shigella dysenteriae* strain NCTC9718 (SDN9) agrees with the findings of Hase-gawa *et al*.(2007), Upton *et al*.(2017) and Al-Fakin and Almagti (2019).

The elution and purification of oleic acid (Yoon *et al*., 2018; Kumar *et al*., 2020; Casillas-vargans *et al*., 2021), 1-docosene (Albralty *et al*., 2023), 1-octadecene (Alawode *et al*., 2023), 14-pentadecenoic acid (Casillas-vargans *et al*., 2021) and E-15-heptadecenal (Afifah *et al*., 2012) agrees with the present study since these fractions were extracted, eluted and purified in the present study

Conclusion

The study revealed presence of *Aspergillus niger* strain CBS513.88 (ANC5), *Aspergillus niger* strain HGH48 (ANH48) and *Aspergillus niger* strain HUS1 (ANH1). The isolates exhibited significant production of potent antibiotics against the enteric bacterial pathogens. The fractions eluted from the fungal isolates showed significant inhibitory activities against enteric bacterial pathogens; *Escherichia coli* strain HH35 (ECH3), *Salmonella enterica* serovar Typhi strain R27 (SETR7) and *Shigella dysenteriae* strain NCTC9718 (SDN9). From the study, ANC5, ANH48 and ANH1 isolated from poultry farms produced significant antibiotics against enteric bacterial pathogens and could be used as alternative antibiotics for nosocomial infections.

Acknowledgments

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

References

Akinfala, T. O., Houbraken, J., Sulyok, M., Adedeji, A. R., Odebode, A. C. and Krska, R. (2020). Moulds and Their Secondary Metabolites Associated with the Fermentation and Storage of Two cocoa Bean Hybrids in Nigeria. *International Journal of Food Microbiology* 316: 108 – 490.

Akinyemi, A. (2017). Antimicrobial activities of secondary metabolites from fungal endophytes. *IOSR Journal of Pharmaceutical and Biological Science* 12(6): 13 – 17

Alawode, T., Lajide, L., Owolabi, B. and Olaleye, M. (2020). Antimicrobial, antioxidant, and GC-MS analysis of hexane extract of the leaves of *Solanum erianthum*. Sixth (6th) International Electronic Conference on Medicinal Chemistry, 1-30 November, 2020, pp. 1-16

Albratty, M., Alhazim, H.A., Meraya, A.M., Najimi, A., Alami, M.S., Rehman, Z. and Moni, S.S. (2023). Spectral analysis and antibacterial activity of the bioactive principles of *Sargassum tenerrimum* j. Kingdom of Saudi Arabia. *Brazilian Journal of Biology* 83: 1 - 10

Al-Shaibani, A.B.A., Al-Shakarchi, F.I. and Ameen, R.S. (2013). Extraction and characterization of antibacterial compound from Aspergillus niger. *Journal of Al-Nahrain University/Science* 16(4): 167 – 174

Amina, B., Sana, G., Atef, J., Laid, D. and Noreddine, K.C. (2017). Antibacterial activity of Aspergillus isolated from different Algerian ecosystem. *African Journal of Biotechnology* 16(32): 1699 – 1704

Ano, Y., Ikado, K., Shindo, K., Koizumi, H. and Fujiwara, D. (2017). Identification of 14-dehydroergosterol as a Novel Anti-inflammatory Compound Inducing Tolerogenic Dendritic Cells. *Science Report Journal* 7:10 – 38

Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N. and Lee, S. Y. (2019). Updates to the Secondary Metabolite Genome Mining Pipeline. *Nucleic Acids Research* 47 (1):81 – 87

Cairns, T. C., Nai, C. and Meyer, V. (2018). How a Fungus Shapes Biotechnology: 100 Years of *Aspergillus niger* Research. *Fungal Biology and Biotechnology* 5:13 – 18

Casillas-Vargas, G., Ocasio-Malave, C., Medina, S., Morales-Guziman, C., Delvaaaaaile, R.G. Carballeira, N.M. and Sanabria-Rios, D.J. (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Prognostic Lipid Reports* 82: 1 - 28

Daood, U., Matinimna, J.P., Pichika, M.R., Mak, K., Nagendrabadu, V. and Fawzy, A.S. (2020). A quaternary ammonium silane antimicrobial triggers bacterial membrane and biofilm destruction. *Scientific Reports* 10: 10970 - 10984

Elissawy, A. M., Ebada, S. S., Ashour, M. L., El-Neketi, M., Ebrahim, W. and Singab, A. B. (2019). New Secondary Metabolites from the Mangrove-Derived Fungus *Aspergillus Sp*. AV-2. *Phytochemistry Letter* 29: 1 – 5.

Fang, W., Lin, X., Wang, J., Liu, Y., Tao, H. and Zhou, X. (2016). Asperpyrone-Type Bis-Naphtho-γ-Pyrones with COX-2-Inhibitory Activities from Marine-Derived Fungus *Aspergillus niger*. *Molecules* 21 (7): 941.

Fukuda, T., Kurihara, Y., Kanamoto, A. and Tomoda, H. (2014). Terretonin G, a new sesterpenoid antibiotic from marine-derived Aspergillus species OPM FOO272. *Journal of Antibiotics* 67(8): 6 7(8): 593 – 595

Iheukwumere, I.H., Dimejesi, S.A., Iheukwumere,C.M., Chude, C.O., Egbe, P.A., Nwaolisa,C.N., Amutaigwe, E.U., Nwakoby, N.E., Egbuna, C., Olisah, M.C. and Ifemeje, J.C. (2020). Plasmid curing potentials of some medicinal plants against citrate negative motile *Salmonella* species. *European Journal of Biomedical and Pharmaceutical Sciences* **7**(5); 40 -47.

Ihekwumere, C.M., Umedum,C.U.and Iheukwumere**, I.H.** (2020). Identities and prevalence of Aspergillus species on *Phaseolus vulgaris* (Bean) seeds sold in Ihiala, Anambra State, Nigeria. *Greener Journal of Microbiology and Antimicrobials* **5**(1): 16 – 25.

Iheukwumere, I. H., Iheukwumere, C. M., Obiefuna, O. H., Unaeze, B. C., Nwike, M. I., Obianom, A. O. and Nwakoby, N. E. (2025). Mycological Medium for the Production of Antibiotics against Urogenital Bacterial Pathogens from Natural Sources. *Journal of Pharmaceutical Research International*, *37*(3), 79-93.

Iheukwumere, I.H. and Umedum, C.U. (2013). Effects of *Gossypium hirsutum* leaf extracts on Gram negative bacteria isolated from the cervix of females with unexplained infertility. *African Journal of Sciences* 14(1): 3261 – 3270.

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research* **8**: 21–33

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Onwuasoanya, U. F., Oduoye, O. T., Ike, V. E., Obiefuna, O. H., Igboanugo, E. U., Ejike, C. C., Udeagbara, O. E., Ochibulu, S. C., Onyemekara, N. N., Ihenatuoha, U. A., Nwakoby, N. E., and Ilechukwu, C. C. (2025c). Structural Elucidation and Antibacterial Evaluation of Natural Products from the Nigric Section of Aspergillus Species against Sorbitol-Positive and -Negative Escherichia coli. *IPS Journal of Natural Products* **1**(1): 1–12.

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A. ., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., and Ilechukwu, C. C. (2025d). Exploring the Phytochemical and Antimicrobial Properties of Fruit Vinegar: A Study on Phoenix Dactylifera and Malus Sylvestris. *IPS Journal of Applied Microbiology and Biotechnology* **4**(1): 115–122.

Ismaiel, A.A., Rabie, G.H. and Abd El-Aal, M.A. (2016). Antimicrobial and morphogenic effects of emodin produced by *Aspegillus awamori* WIRI20. *Biologia* 7(6): 464 – 474

Kumar, P., Lee, J.M Beyenal, H. and Lee, J. (2020). 'Fatty acids as antibiofilm and antivirulence agents. *Trends in Microbiology* 28(9): 753 - 758

Kwaguchi, M., Nonaka, K. and Tomoda, H. (2013). New method for isolating antibioticproducing fungi. The Journal of Antibiotics 66: 17 – 21.

Kwoseh, C.K., Asomani, D. and Adubofour, K. (2012). Cassava starch-agar blend alternative gelling agent for mycological culture media. *Journal of Agriculture and Applied Sciences* 8(1): 3 – 8

Oledibe, O.J., Enweani-Nwokelo, I.B., Okigbo, R.N. and Achugbu, A.N. (2023). Formulatiob of fungal media from local plant materiala. Advanced Gut and Microbiome Research Journal 1: 20 – 23.

Rao, V.K., Ramana, M.V., Girisham, S. and Reddy, S.M. (2012). Influence of carbon and nitrogen sources on ochratoxin A production by two species of Penicillium isolated from poultry feeds. National Academy Science Letters 36(1): 1010 – 110

Simin, H.N. (2011). "Sonicated date syrup media preparation for microbial culture,"*African Journal of Biotechnology* 10 (3): 424–432

Tharmila, S., Jeyaseelam, E.C. and Thavaranjit, A.C. (2011). Preliminary screening of alternative culture media for the growth of some selected fungi. Archives of Aplied Scientific Research 3(3): 389 – 393

Yahya, A.M.., Salim, A.B. and Hmoshi, R.M. (2022). Isolation and identification of Penicillium rubens from the local strain in Mosul, Iraq and investigation of potassium phosphate effect on its growth. Archives of Razi Institute 77 (1): 421 – 427

Yoon, B., Jackman, J., Vall-Gonzallez, E. and Cho, N. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. *International Journal of Molecular Science* 19(4): 1114