ORIGINAL ARTICLE

Antimicrobial and Cytotoxic Activities of *Nigrospora oryzae* Endophytic Fungus Isolated from *Moringa oleifera*

[1]Aml Ghanem, [2]Ahmed A. Al-Karmalawy, [3]Noha E. Morsy, [4]Mahmoud Elsabahy, [5]Ahmed M. Rayan*

[1]School of Biotechnology, Badr University in Cairo, Badr City, Cairo 11829, Egypt; Institute of Biotechnology for Postgraduate Studies and Research, Suez Canal University, Ismailia 41522, Egypt

[2]Department of Pharmaceutical Chemistry, College of Pharmacy, The University of Mashreq, Baghdad 10023, Iraq; Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Horus University-Egypt, New Damietta 34518, Egypt

[3]Department of Food Science & Technology (Home Economics Branch), Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt

[4]Badr University in Cairo Research Center, Badr University in Cairo, Badr City, Cairo 11829, Egypt

[5]Institute of Biotechnology for Postgraduate Studies and Research, Suez Canal University, Ismailia 41522, Egypt; Department of Food Technology, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt

Key words:
Endophytic fungi, Molecular identification, Cytotoxicity assay, Antimicrobial activity, Pharmacological applications

Corresponding Author:
Ahmed M. Rayan
Department of Food Technology, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt
Tel.: 01099968554
ahmed_rayan@agr.suez.edu.eg
ORCID: https://orcid.org/0000-0003-3544-4675

ABSTRACT

Background: *Endophytic fungi are known to produce bioactive compounds with potential therapeutic applications.* ***Objective****: This study investigated the antimicrobial and cytotoxic activities of Nigrospora oryzae, an endophytic fungus, isolated from Moringa oleifera.* ***Methodology:*** *Nigrospora oryzae was cultured, and its secondary metabolites were extracted and screened for antimicrobial efficacy against a range of pathogenic microorganisms, including Bacillus subtilis ATCC 6051, Escherichia coli ATCC 8739, Klebsiella pneumoniae NCTC 13465, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10231.* ***Results:*** *The results demonstrated that extracts from Nigrospora oryzae exhibited high antimicrobial activity against Staphylococcus aureus and Candida albicans compared to other microorganisms. The cytotoxicity assays revealed a dose-dependent inhibition of cell proliferation in MDA-MB-231 cell lines, suggesting potential anticancer properties. IC50 values indicating moderate cytotoxicity for DCM extract (44.66 µg/ml) and superior efficacy for Doxorubicin (40.79 µg/m). In silico docking validation of AutoDock Vina showed reliable RMSD values for DNA Gyrase B, DNA Topoisomerase 4, and PARP3, supporting the in vitro results. Among the identified compounds, Aurofusarin demonstrated the most promising inhibitory potential, with high binding affinities across all tested proteins (-11.44 to DNA Gyrase B, -8.40 to DNA Topoisomerase 4, and -11.55 to PARP3). While Mellein and Nigrospin showed lower activity, Aurofusarin's strong binding suggests significant pharmacological potential.* ***Conclusion:*** *This study highlights the antimicrobial and anticancer potential of Nigrospora oryzae extracts, particularly Aurofusarin, as a less toxic alternative to chemotherapy drugs like Doxorubicin. Further research should focus on its mechanisms of action and potential use in oncological therapies.*

INTRODUCTION

Moringa oleifera "miracle tree" has gained significant attention in recent years due to its numerous nutritional and medicinal properties[1], that widely distributed across tropical and subtropical regions worldwide[2], it is particularly valued for the nutritional content of its leaves. The leaves are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, tannins, and saponins[3,4]. Moreover, it serves multifaceted purposes, finding applications in food, medicine, cosmetic oil production, and even as a valuable forage option for livestock[4]. Endophytes that are living in *Moringa oleifera* without causing any apparent harmful effects can enrich it with bioactive compounds and increase its pharmacological activity. Twenty-four endophytic fungi were discovered in *Moringa oleifera* leaves, identified within the genera Fusarium, Xylaria, Pestalotiopsis, *Aspergillus*, *Stachybotrys*, *Rhizoctonia*, *Macrophomina* and *Nigrospora oryzae*[5], they have been explored and suggested to produce novel bioactive compounds[6]. These compounds may have pharmaceutical properties, such as antimicrobial, anticancer and antioxidant activities[7]. *Nigrospora oryzae* is a promising endophytic fungus that has the potential to produce a wide range of bioactive compounds with significant therapeutic value[8]. Further

research is needed to fully characterize these compounds, explore their mechanisms of action, and develop them into novel therapeutic agents[9]. By harnessing the potential of *Nigrospora oryzae*, researchers have unlocked new avenues for the discovery and development of natural products with applications in medicine, agriculture, and other industries[10].Therefore, this study focuses on isolating metabolites produced by *Nigrospora oryzae*, endophytic fungus, in *Moringa oleifera* and evaluating their biological activity.

METHODOLOGY

Plant sample collection and identification

A fresh and healthy leaves of *Moringa oleifera* plant were collected in a sterile polyethylene bag from the medicinal garden at Badr University in Cairo, Egypt, at August 2022. The samples were kindly identified at the Department of Pharmacognosy, Faculty of Pharmacy, Badr University, Cairo, Egypt, then subjected to an endophytic fungi isolation process freshly within 5 h of collection.

Isolation of endophytic fungi

Leaves were washed with tap water, air-dried, and surface-sterilized with 95% ethanol for 30 s, 5% sodium hypochlorite for 5 min, 95% ethanol for 30 s, and rinsed with sterile distilled water for 3–5 s. Sterile surgical blades were used to cut leaves into 1 × 1 cm^2 segments. To inhibit bacterial growth, these segments were placed on PDA medium supplemented with 340 µL of Gentamicin (5 mg/10 mL) in 250 mL of medium. Petri dishes were sealed and incubated at 27℃ ± 2℃ for 5 days, with daily monitoring. Emerging hyphal tips were sub-cultured onto fresh PDA without antibiotics to ensure purity.

Molecular identification of endophytic fungi

Fungal isolates were identified morphologically by colony color, texture, and diameter at Badr University, Cairo. Molecular identification using ITS gene sequencing was conducted by Macrogen Inc., Seoul, and sequences were aligned and analyzed via BLAST (NCBI). Identified sequences with 99% ITS similarity were deposited in GenBank, with species classified

Phylogenetic analysis

The obtained data from Macrogen Inc. was aligned using MEGA X software. The search for homologous sequences was done using Basic Local Alignment Search Tools (BLAST) at the National Center for Biotechnology Information online (https://www.ncbi.nlm.nih.gov/). All fungal endophyte sequences with 99–100% similarities had the best hit in the NCBI database.

Crude extract preparation of endophytic fungus *Nigrospora oryzae*

Mass growth production for the endophytic fungus *Nigrospora oryzae*, 10 flasks with rice medium were incubated in the dark for 21 days at room temperature. After incubation, 250 mL of ethyl acetate was added to each flask, and extracts were concentrated under vacuum at 45°C, yielding 50 g of crude extract. Liquid-liquid fractionation was then performed using a series of solvents (n-hexane, DCM, EtOAc, and MeOH), resulting in four distinct fractions: n-hexane (H), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH).

Liquid Chromatography-Mass Spectrometry (LC-MS)

Chemical profiling of the fungal constituents was performed using LC-MS, a Triple quadruple instrument (C-18 column 1.7 µm particle size - 2.1 × 50 mm) (XEVO TQD, Waters Corporation, Milford, USA) at the Center for Drug Discovery, Research and Development (CDDRD), Faculty of Pharmacy, Ain Shams University.

The minimum inhibitory concentration MIC Test

The MIC method was used to assess the anti-bacterial activities of *Nigrospora oryzae* DCM fraction against *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* NCTC 13465., *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC10231[11]. In brief, DCM fraction containing *Nigrosora oryzea* metabolites and gentamycin were dissolved in a bacterial growth medium separately and adjusted to different concentrations by double dilution. 100 µL of bacterial suspension and the fraction were separately added to a 96-well plate for 24 h of incubation, the control group was added with an equal amount of bacterial growth medium. Then, 20 µL of 0.2% 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was added to each well. After 4 h of reaction, the mixture color was observed, and the fraction concentration at which the red precipitate in the well was not visible, was considered as the MIC.

MTT- Cytotoxicity assay

The cytotoxicity of the DCM fraction was tested against MDA-MB-231 breast cancer cells, was obtained from (VACSERA, Research Dept, Cairo, Egypt). Doxorubicin was used as a reference drug. Cells (4×10^4 cells/mL) were plated in DMEM with 10% FBS and incubated at 37°C, 7% CO_2. After 20 hours, doses of 50, 25, 12.5, and 6.25 µg/mL of test compounds were added. Controls included untreated wells and media-only blanks. After 48 hours, cells were washed, treated with MTT solution, and incubated for 4 hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at

570 nm. Cell proliferation ratios were calculated, and IC50 values were used to determine antitumor activity.

***In silico* molecular screening studies**

At first, target proteins were identified by investigating common cellular pathways amongst various bacterial strains using STRING Database. An inclusive selection of gram-positive and gram-negative targeted proteins (DNA Gyrase – Subunit B and DNA Topoisomerase 4) was considered to investigate the extracts potential activity as antibacterial agents. As for their potential to act as anticancer agents in breast cancer, the essential mechanism of DNA repair was targeted through Poly (ADP-Ribose) Polymerase-3 (PARP3). Then, Protein Data Bank entries used for molecular screening (3G7E [DNA Gyrase – Subunit B] – 4KOE [DNA Topoisomerase 4] – 4L7O [PARP3]) were chosen based on their integrity, quality, and accommodability from available entries on UniProt Database. Afterwards, docking of extract molecules on target proteins was performed by AutoDock Vina (v1.1.2). Eventually, docking parameters were set the same for all proteins (exhaustiveness = 16 & number of modes = 9 (default)) except for the grid box at the active site; it was set to be a relaxed fit for pocket residues at 10.1Å, 11.8Å, 21.3Å for DNA Gyrase – Subunit B (3G7E), 15.3Å, 17.1Å, 10.9Å for DNA Topoisomerase 4 (4KOE), and 17.8Å, 17.4Å, 10.6Å for PARP3 (4L7O). Also, all boxes were centered on pocket-accommodatable space. Eventually, best binding poses with highest binding affinity for each extracted compound were selected, analyzed, and reported.

Statistics Analysis

The data were collected from three independent experiments and analysed using One-Way ANOVA by Graph Pad Prism. Data are expressed as mean ± SD of experiments performed in triplicate. Error bars represent the SD and $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$, significant difference compared to the control.

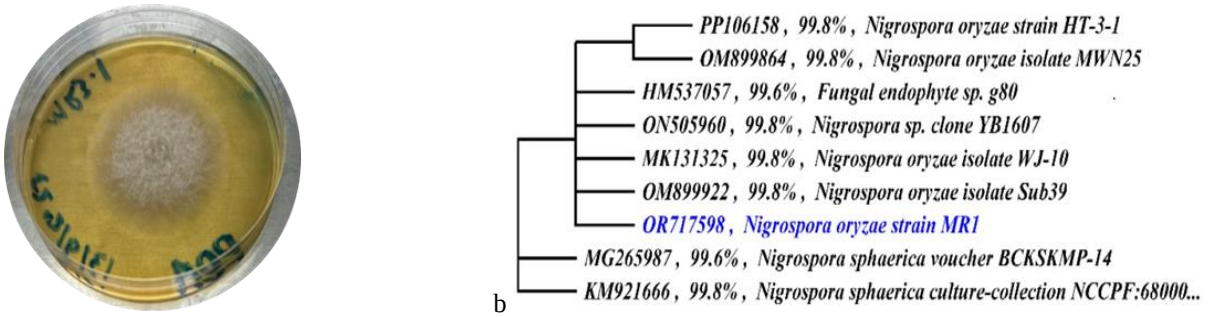
RESULTS

Isolation and identification of endophytic fungi

Endophyte isolation was done under extremely sterile condition, the plates were checked to make sure the growth that was seen originated from the inside of the samples. Culture of *Moringa oleifera* leaf fragments on (PDA) plates reveals developing mycelia of endophytic fungus *Nigrospora oryzae* after 6 days of incubation and the ensuing pure culture of that fungus obtained after subculture on a new PDA plate Fig. 1a.

Phylogenetic analysis of OR717598 *Nigrospora oryzae*

The phylogenetic tree shows that the *Nigrospora oryzae* strain MR1 (OR717598) clusters closely to other *Nigrospora oryzae* isolates, such as strains HT-3-1 (PP106158) and MWN25 (OM899864), all of which show high sequence similarity (99.8%). This close clustering supports the identification of strain MR1 as *Nigrospora oryzae.* Strain MR1 (OR717598) and other *Nigrospora oryzae* strains are distinct from other *Nigrospora* species such as *Nigrospora sphaerica* (MG265987, KM921666), which show slightly lower similarity percentages (99.6% and 99.8%). This differentiation supports the specificity of the sequences used for species identification and highlights the genetic diversity within the *Nigrospora* genus (Fig. 1b).



a b

Fig. 1. (a) Endophytic fungus (*N. oryzae*). (b) Neighbor-joining (NJ) phylogenetic tree based on ITS-rDNA sequences of endophytic fungi associated with the *N. oryzae*. Numbers above or below branches indicate bootstrap values of NJ and MP analyses (> 50%, right) from 1000 bootstrap replicates).

LC-MS analysis of *Nigrospora oryzae* dichloromethane (DCM) fraction

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis was performed to identify the secondary metabolites of *N. oryzae* DCM fraction. Three known compounds were identified as (1) Mellein, (2) Nigrospin C and (3) Aurofusarin as seen in Table (1).

Table 1: The identified compounds of *N. oryzae* dichloromethane (DCM) fraction

SN	Name	Formula	Mass	Rt	ESI (*m/z*)		Ref
					M+H	M-H	
1	Mellein	$C_{10}H_{10}O_3$	178.185	11.24	179.1054	-	[12]
2	Nigrospin C	$C_{18}H_{26}O_5$	322.396	12.62	-	321.2969	[13]
3	Aurofusarin	$C_{30}H_{18}O_{12}$	570.457	13.89	571.2708	-	[14]

Antimicrobial activity of *N. oryzae* DCM fraction

The Minimum Inhibitory Concentration (MIC) of the DCM extract from *Nigrospora oryzae* was evaluated against a range of bacterial strains, including *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* NCTC 13465, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 (Fig. 2). The MIC values for the DCM extract of *Nigrospora oryzae* varied among the tested strains, ranging from 12.3 µg/mL to 21.1 µg/mL. The highest MIC was observed against *K. pneumoniae* NCTC 13465 (21.1 µg/mL), while the lowest was against *E. coli* ATCC 8739 (12.3 µg/mL). In comparison, Gentamycin displayed MIC values ranging from 13.5 µg/mL to 23.6 µg/mL, with the highest MIC also against *K. pneumoniae* NCTC 13465 (23.6 µg/mL) and the lowest against *B. subtilis* ATCC 6051 (13.5 µg/mL). The DCM extract demonstrated considerable inhibitory effects on *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853, with MIC values of 15.5 µg/mL and 18.4 µg/mL, respectively. These results were slightly lower than the MIC values observed for Gentamycin, which were 17.7 µg/mL for *S. aureus* and 14.8 µg/mL for *P. aeruginosa*. The standard deviations across the triplicate measurements were relatively low, indicating consistent results. The standard deviation for the DCM extract ranged from 0.17 to 0.49, while for Gentamycin, it ranged from 0.25 to 1.52.

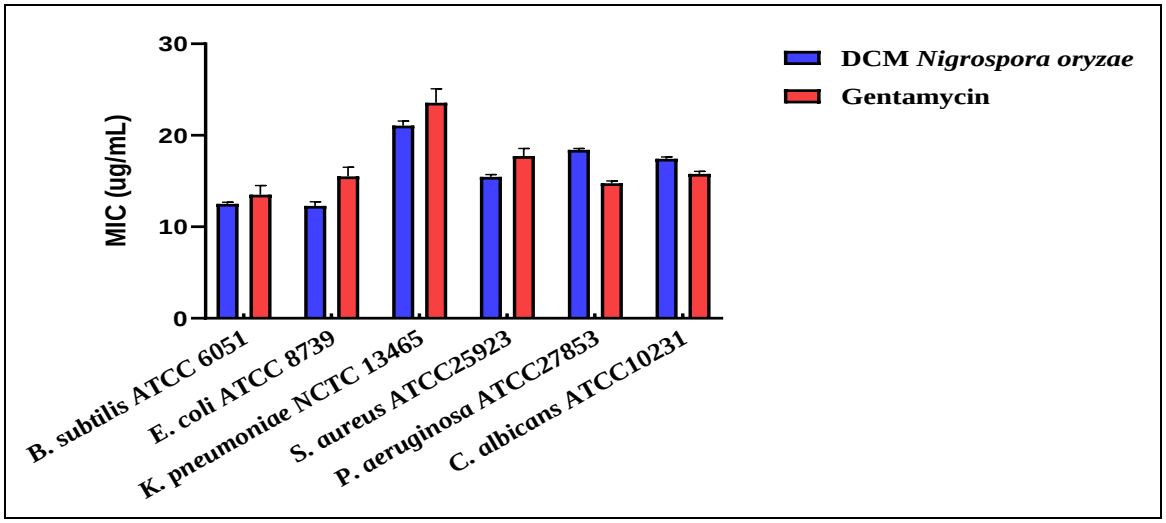


Fig. 2: Comparative antimicrobial activity of DCM extract from *N. oryzae* and Gentamycin.

Cytotoxic effect of *N. oryzae* DCM fraction

The DCM extract of *N. oryzae* demonstrates high efficacy at lower concentrations (0 to 0.9 log), maintaining inhibition percentages above 99%, and shows a significant drop-in activity from 1.5 to 3.0 log concentration units, where the inhibition percentage decreases from 70.12% to 4.79%, with consistently low standard deviation (SD) indicating reliable performance (Fig. 3a). Conversely, Doxorubicin presents a steady decline in efficacy as concentration increases, starting from 100.73% at 0 concentration to 0% at 3.0 log concentration units, with a more gradual decline compared to the DCM extract, noticeable from 1.2 log concentration units onwards: additionally, the standard deviation values for Doxorubicin were low with increasing concentrations, indicating higher variability at lower concentrations. The bar graph of IC50 values further supports these observations, revealing that the IC50 for the DCM extract is 44.66 µg/ml, indicating moderate cytotoxicity, whereas Doxorubicin has a significantly lower IC50 40.79 µg/ml, reflecting its superior efficacy in inhibiting cell viability at lower concentrations (Fig. 3b).

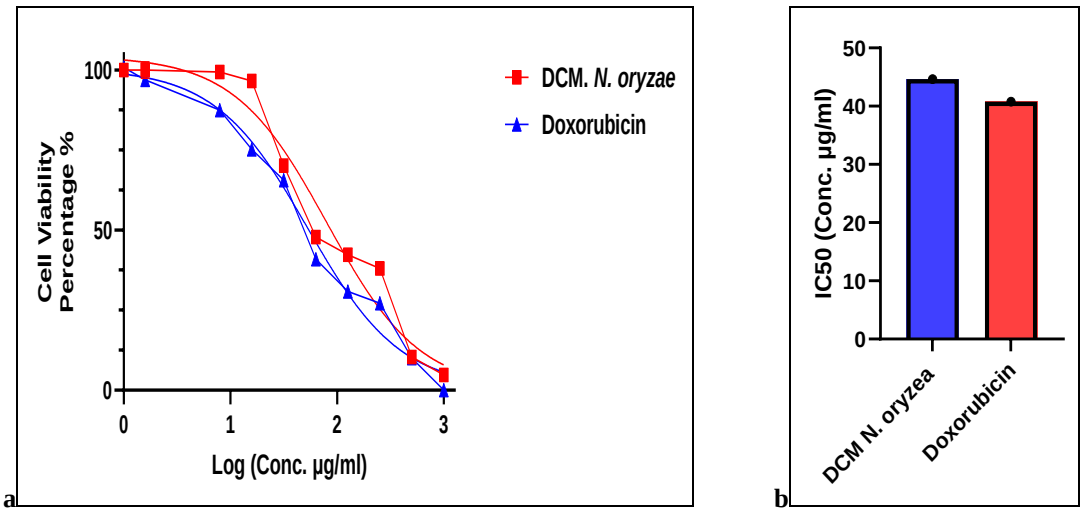


Fig. 3: Anti-cancer activity of *N. oryzea* DCM fraction

***In-silico* metabolites prediction and molecular screening studies**

Docking validation of AutoDock Vina was first performed using visual inspection and Root-Mean-Square Deviation (RMSD) calculation for each co-crystallized ligand of target proteins (Fig. 4).

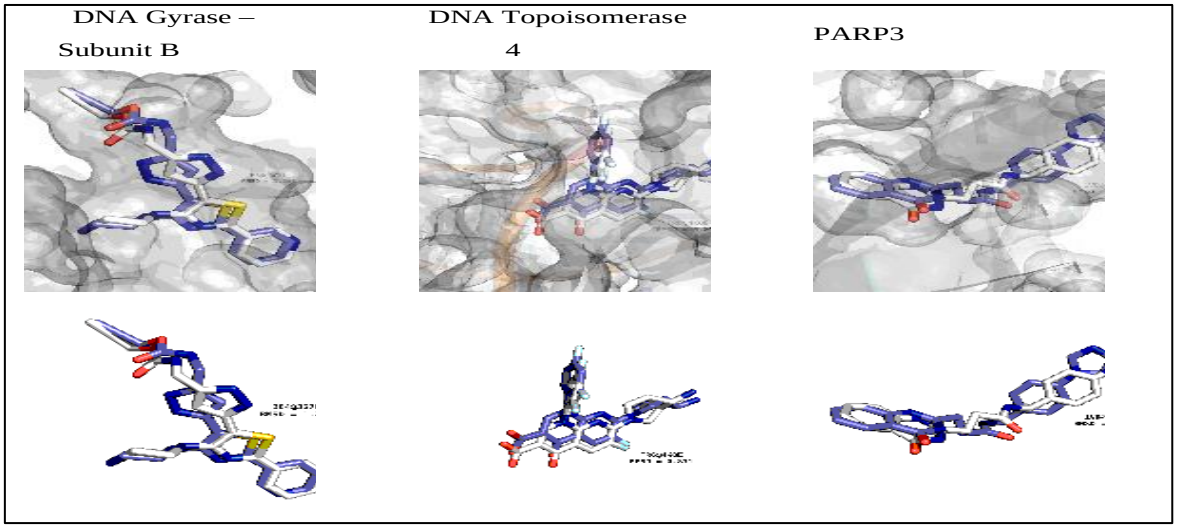


Fig. 4: Docking Algorithm validation indicated by RMSD values using AutoDock Vina. Docked co-crystallized ligand (Violet) superimposed on imaged co-crystallized ligand (White)

The resulted RMSD scores were found to be within acceptable range (<2.5) (Table 2). Only three prominent compounds were found to have descent yield of *N. oryzae* extract. However, Aurofusarin only shows promising potential to have an inhibitory activity on target proteins. Other compounds like Mellein and Nigrospin may show certain levels of activity on one or more of target proteins.

Table 2. RMSD values for co-crystallized ligands of target enzymes.

Protein	CCL Code	Conformer	RMSD
DNA Gyrase B	B64	2	1.298
DNA Topoisomerase 4	TR6	1	0.871
PARP3	1VD	1	1.987

DNA Gyrase – Subunit B (PDB: 3G7E)

Quinolones are known to be effective inhibitors of DNA Gyrase. However, their best activity could be seen on DNA Gyrase – Subunit A, yet they show a certain level of potential activity on Subunit B. The active site on DNA Gyrase B consists of multiple interacting residues each of which is responsible for certain interaction properties. The essential interaction for the protein to be rendered inactive involves Aspartate-73 on chain A (Fig 5). However, interactive carboxyl group of Aspartate-73 is directed to a tight angle parallel to ligands' orientation, which make it necessary to overcome this issue by interacting with ligands over a hydrogen bridge formed by a nearby water molecule.

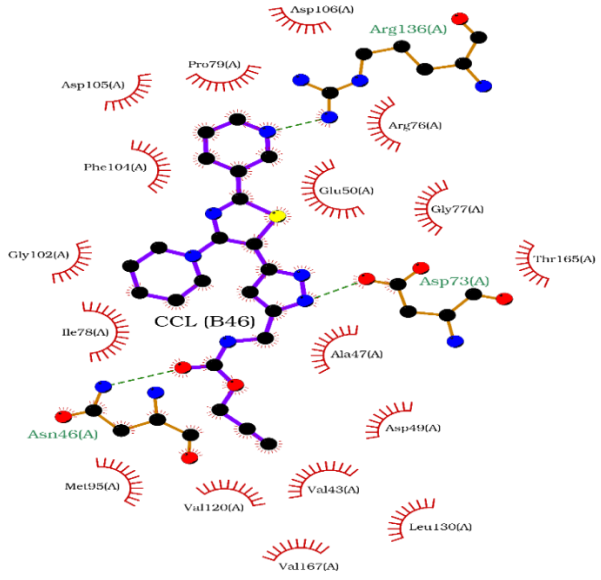


Fig. 5. Flat (2D) representation illustrates the hydrophilic and lipophilic interaction between DNA Gyrase – Subunit B and its Co-Crystallized Ligand.

DNA Topoisomerase 4 (PDB: 4KOE)

One special feature of DNA Topoisomerase 4 active site is its flexibility to be inhibited by immobilizing one or both residues of Arg-117 and Tyr-118 since both are bonded together and are required to be bound to a ligand to inhibit cleavage of DNA backbone. One more feature of DNA Topoisomerase 4 is the persistent Magnesium ion close to Ser-79 (Fig. 6). This ion contributes to maintaining the active site of the protein hydrogen-thirst by attracting the lone pair of nearby protein oxygen's, which allows better affinity for interacting, hydrogen-donor molecules.

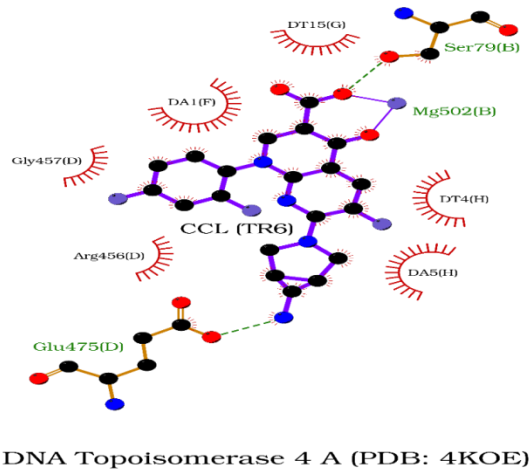


Fig. 6. Flat (2D) representation illustrates the hydrophilic and lipophilic interaction between DNA Topoisomerase 4 and its Co-Crystallized Ligand.

Poly (ADP-Ribose) Polymerase-3 (PARP3) (PDB: 4L7O)

PARP3 is characterized by a special lipophilic interaction with Tyr-425 that boosts the affinity of the inhibitory agent when bound properly. However, without the essential interaction to Gly-385, no inhibitory activity would be significant. Favorable hydrophilic interaction with Asp-387 and lipophilic interaction with Tyr-414 could result in enhanced affinity to PARP3 (Fig. 7). Yet, hydrophilic interaction with Asp-387 could be facilitated through the participation of a water molecule to form a hydrogen bridge between the inhibitory molecule and the Asp-387. Exclusively, Arg-400 may contribute to an increased potency for inhibitory molecules that would bind to it.

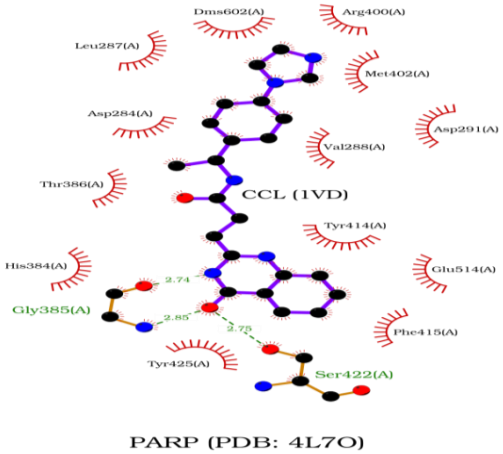


Fig. 7. Flat (2D) representation illustrates the hydrophilic and lipophilic interaction between Poly(ADP-Ribose) Polymerase-3 (PARP3) and its Co-Crystallized Ligand.

Table 3 illustrates the calculated affinities for our subjects of investigation to targeted proteins. Current treatment in market (Doxorubicin, Irinotecan, and Topotecan), and co-crystallized ligand (CCL) with Poly(ADP-Ribose) Polymerase-3 (PARP3) (PDB: 4L7O). Exquisitely, Aurofusarin form multiple interactions with the active site of PARP3 through which it would exhibit a powerful inhibitory activity. Another interaction increasing the potency of Aurofusarin is its interaction with Arg-400 (Table 3).

Table 3: Selected extract compounds affinity to target proteins. ("-": Not Tested, "NA": No Affinity)

Compound	Affinity to Protein		
	Antimicrobial Activity		Anticancer Activity
	DNA Gyrase B	DNA Topoisomerase 4	PARP3
Co-Crystallized Ligands	-9.74	-11.40	-9.13
Amoxicillin	-5.82	NA	-
Ciprofloxacin	-7.68	NA	-
Clavulanic Acid	-6.08	-5.97	-
Moxifloxacin	-6.82	-11.12	-
Norfloxacin	-7.32	NA	-
Doxorubicin	-	-	-10.90
Irinotecan	-	-	-4.23
Topotecan	-	-	-4.85
Aurofusarin	-11.44	-8.40	-11.55
Mellein	NA	NA	-7.43
Nigrospin	-7.10	NA	-7.35

DISCUSSION

Endophytic fungi produce a wide variety of chemical compounds, such as terpenes, alkaloids, monoterpenoids, peptides, and polyketides, that have recently received a lot of attention in drug development due to their abundance and ubiquity[15, 16]. *Moringa oleifera* is a traditional medicinal plant, that widely utilized for its extensive nutritional and medicinal values[17, 18]. *Nigrospora oryzea* fungus was isolated from the leaf part of the plant and identified morphologically and genetically, using its DNA barcode that used to identify the diversity of the fungi then it was deposited in the gene bank under accession number OR7171595, code MR1 and selected for further studies. The investigation of the DCM fraction of *N. oryzea* extract resulted in the identification of three active compounds. Their structures were determined using powerful analytical technique LC-MS for *identification* of compounds, their biological activities were subsequently evaluated. These compounds namely (1) Mellein, (2) Nigrospin C and (3) Aurofusarin. The first identified compound Mellein which is known for its phytotoxic effect and antimicrobial properties[19, 20]. Nigrospins C was reported to exhibit an inhibition zone diameter of 8 cm at a concentration of 50 µg/disc against *A. versicolor*[21]. In our study, the DCM extract of *Nigrospora oryzae* exhibited broad-spectrum antimicrobial activity, as indicated by its MIC values across the six tested microbial strains. The effectiveness of the extract was particularly notable against *K. pneumoniae* NCTC 13465 and *S. aureus* ATCC 25923, both of which are important pathogens associated with human infections. The MIC values for the DCM extract were generally comparable to those of Gentamycin, the extract showed slightly lower activity against *P. aeruginosa* ATCC 27853. This could suggest that the bioactive compounds present in the *Nigrospora oryzae* extract are more effective against Gram-positive bacteria than Gram-negative bacteria. Compounds such as Mellein, Nigrospin C, and Aurofusarin, identified in the DCM extract, are likely critical contributors to its antimicrobial activity. Mellein is known for its broad-spectrum antimicrobial properties, which probably contributed to the observed inhibition zones. Nigrospin C and Aurofusarin also possess antimicrobial characteristics, with Aurofusarin particularly noted for its activity against fungi like *C. albicans* (17.2-17.6 mm)[22]. The presence of these bioactive compounds suggests that while the DCM extract may not entirely match Gentamycin's efficacy, its natural constituents offer a promising alternative for antimicrobial agents. This warrants further investigation to optimize its potency and application spectrum[23-25]. The lower standard deviations in the measurements indicate that the antimicrobial activity of the DCM extract is consistent and reproducible. This consistency supports the potential of the extract as a reliable natural antimicrobial agent. Comparing

the MIC values of the DCM extract to Gentamycin, it is evident that while the extract is effective, Gentamycin still exhibits stronger inhibitory effects in some cases. However, given the rise in antibiotic resistance, the use of natural products like the *N. oryzae* extract could provide a complementary approach to traditional antibiotics. Future research should focus on isolating and identifying the specific compounds responsible for the antimicrobial activity in the *N. oryzae* extract. Additionally, studies on the synergistic effects of the extract with conventional antibiotics could provide valuable insights into new therapeutic strategies against resistant strains. The MTT assay results demonstrate the cytotoxic effects of *N. oryzae* DCM extract and Doxorubicin on MDA-MB-231 cell line. The comparative analysis between the DCM extract of *N. oryzae* and Doxorubicin reveals distinct pharmacodynamic profiles, with each compound displaying unique patterns of efficacy across various concentration ranges. The DCM extract exhibits optimal performance at lower concentrations (0 to 0.9 log), maintaining inhibition percentages above 99%, indicative of its potent activity. However, this efficacy substantially diminishes between 1.5 to 3.0 log concentration units, where inhibition drops sharply from 70.12% to a mere 4.79%, while consistently low standard deviation values underscore the extract's reliable performance across replicates. In contrast, Doxorubicin demonstrates a continuous and more gradual decline in efficacy over increasing concentrations, starting with an inhibition rate of 100.73% at 0 log concentration and tapering off to 0% at 3.0 log concentration units. Notably, this gradual decline begins from 1.2 log concentration units, signifying a steadier reduction in activity compared to the abrupt drop observed with the DCM extract. Additionally, Doxorubicin's standard deviation values decrease with higher concentrations, highlighting greater variability at lower doses. The bar graph illustrating IC50 values further corroborates these findings, with an IC50 for the DCM extract at approximately 44.66 µg/ml, signifying moderate cytotoxicity, whereas Doxorubicin achieves a markedly lower IC50 of around 40.79 µg/ml, underscoring its superior inhibitory potency at reduced concentrations. This comparative data provides critical insights into the efficacy and cytotoxic profiles of both compounds, guiding their potential therapeutic applications and dosing strategies. These findings suggest that DCM of *N. oryzae* extract has potential as an anticancer agent[26]. Further investigation through molecular screening resembled a better understanding of potential mechanism of action of extract compounds as anticancer and antimicrobial agents. Molecular screening revealed Aurofusarin as the primary molecule of potential in the extract. With its affinity to DNA Gyrase B higher than commonly used market medications for gram negative bacteria[27], Aurofusarin represents a potent antimicrobial agent. Additionally, Aurofusarin interaction with DNA Topoisomerase 4 outstands its potential as an antimicrobial agent compared to Amoxicillin and Ciprofloxacin[28]. As for the extract, these inhibitory properties are further advanced by the minor potential of Nigrospin as a DNA Gyrase B inhibitor. On the other side, extract compounds have a high potential of inhibition for PARP3; which is a major beneficiary pathway for breast cancer to develop further. However, Aurofusarin was found to be the major player in the inhibitory activity against PARP3 and even outcoming the potency of Doxorubicin.

CONCLUSION

This study confirms the novel antimicrobial and anticancer effectiveness of Mellein, Nigrospin c and Aurofusarin of *N. oryzae* extract, demonstrates their ability to inhibit a variety of pathogens at different concentrations, and they also have cytotoxic activity on cancer cell line. Future research should ensure molecular screening results of the antimicrobial and anticancer mechanism of action to clarify its therapeutic applications, potential strategies for mitigating resistance, and the potential of Aurofusarin as an adjuvant therapy in breast cancer. The comparative analysis between DCM of *N. oryzae* extract and Doxorubicin on cell viability underscores the potential of fungal extracts as alternative cancer treatments. With further research, *N. oryzae* could help develop new oncological therapies that offer efficacy with controlled cytotoxicity. This study lays the groundwork for further exploration of *N. oryzae* pharmacological potential. The findings suggest that DCM *N. oryzae* extract could be a less aggressive and potentially safer alternative to traditional chemotherapy drugs like Doxorubicin. Although it is less potent, the extract's higher IC50 value indicates it might be used at higher doses without reaching toxic levels, balancing efficacy with patient quality of life. These results support continued pharmacological research into *N. oryzae* extracts, focusing on identifying active compounds and elucidating their mechanisms of action.

Conflict of interest: The authors declared no conflict of interest.

This manuscript has not been previously published and is not under consideration in another journal.

Availability of data and material: Data are available upon request.

Funding: Authors did not receive any grants from funding agencies.

REFERENCES

1. Palada MC, Webert AW, and Joshi RC (Eds.). The Miracle Tree: *Moringa oleifera*. 2nd ed. Iloilo City: Central Philippine University, 2019.
2. Rajbhar YP, Rajbhar G, Rawat P, et al. Grow Moringa (*Moringa oleifera*), the miracle tree on the earth. HIJ. 2018;2(4):166-72.
3. Shivangini P, Mona K, Nisha P. Comprehensive Review: Miracle Tree *Moringa oleifera* Lam. Curr Nutr Food Sci. 2022;18(2):166-80.
4. Hamza TA, Azmach NN. The miraculous moringa trees: From nutritional and medicinal point of views in tropical regions. J Med Plants Stud. 2017;5(4):151-62.
5. Zaini M. Isolation and identification of Endophytic Fungi of Pandanus sp. and Alpinia sp. from Reserve Forest UiTM Negeri Sembilan. J Acad. 2019;7(2):124-9.
6. Rehman B, Khan SA, Hamayun M, et al. Potent Bioactivity of Endophytic Fungi Isolated from Moringa oleifera Leaves. Biomed Res Int. 2022 (1); 2461021.
7. Nischitha R, Shivanna M. Diversity and in silico docking of antibacterial potent compounds in endophytic fungus *Chaetomium subaffine* Sergeeva and host *Heteropogon contortus* (L.) P. Beauv. Process Biochem. 2022;112:124-38.
8. Vig R, Bhadra F, Gupta SK, et al. Neuroprotective effects of quercetin produced by an endophytic fungus *Nigrospora oryzae* isolated from Tinospora cordifolia. J Appl Microbiol. 2022;132(1):365-80.
9. Singh A, Singh DK, Kharwar RN, et al. Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: Insights, avenues, and challenges. Microorganisms. 2021;9(1):197.
10. Hashem AH, Attia MS, Kandil EK, et al. Bioactive compounds and biomedical applications of endophytic fungi: a recent review. Microb Cell Fact. 2023;22(1):107.
11. Jin M, Piao X, Wu X, , et al. Oplopanax elatus adventitious root production through fed-batch culture and their anti-bacterial effects. Plant Cell, Tissue and Organ Culture (PCTOC). 2020;140:447-57.
12. Chacón-Morales P, Amaro-Luis JM, Bahsas A. Isolation and characterization of (+)-mellein, the first isocoumarin reported in Stevia genus. Avances en Quimica. 2013;8(3):145-51.
13. Liu J, H Jung J, Liu Y. Antimicrobial compounds from marine invertebrates-derived microorganisms. Curr Med Chem. 2016;23(25):2892-905.
14. Alsarrag A, Alhussein M, Karlovsky P. Aurofusarin analysis by HPLC-MS/MS: challenges of limited solubility and stability of standards in water-containing solvents. Fungal Secondary Metabolites: Fusarium spp. and Microdochium spp. 2021:46.
15. Ariffin SA, Davis P, Ramasamy K. Cytotoxic and antimicrobial activities of Malaysian marine endophytic fungi. 2011; 95 - 100.
16. Katoch M, Singh G, Sharma S, et al. Cytotoxic and antimicrobial activities of endophytic fungi isolated from *Bacopa monnieri* (L.) Pennell (Scrophulariaceae). BMC Complement Altern Med. 2014;14:1-8.
17. Dhanalakshmi R, Umamaheswari S, Sugandhi P, et al. Biodiversity of the endophytic fungi isolated from *Moringa oleifera* of Yercaud hills. Int. J Pharm Sci Res. 2013;4(3):1064.
18. Zhao J, Zhang Y, Wang L, et al. Bioactive secondary metabolites from Nigrospora sp. LLGLM003, an endophytic fungus of the medicinal plant Moringa oleifera Lam. World J Microbiol Biotechnol. 2012;28:2107-12.
19. Abonyi DO, Eze PM, Abba CC, et al. Biologically active phenolic acids produced by Aspergillus sp., an endophyte of *Moringa oleifera*. Eur J Biol Res. 2018;8(3):157-67.
20. Reveglia P, Masi M, Evidente A. Melleins—Intriguing Natural Compounds. Biomolecules. 2020;10(5):772.
21. Dong JJ, Bao J, Zhang XY, et al. Alkaloids and citrinins from marine-derived fungus *Nigrospora oryzae* SCSGAF 0111. Tetrahedron Lett. 2014;55(16):2749-53.
22. Selim KA, El-Beih AA, AbdEl-Rahman T, et al. (2012). Biology of endophytic fungi. Curr Res Environm Appl Mycol, 2012; 2(1), 31-82.
23. Kupnik K, Primožič M, Kokol V,et al.. Enzymatic, Antioxidant, and Antimicrobial Activities of Bioactive Compounds from Avocado (*Persea americana* L.) Seeds. Plants. 2023;12.
24. Octarya Z, Nugroho TT, Nurulita Y, et al. Molecular Identification, GC-MS Analysis of Bioactive Compounds and Antimicrobial Activity of Thermophilic Bacteria Derived from West Sumatra Hot-Spring Indonesia. HAYATI J Biosci. 2022; 29(4), 549-561.
25. Šojić B, Milošević S, Savanović D, et al. Isolation, Bioactive Potential, and Application of Essential Oils and Terpenoid-Rich Extracts as Effective Antioxidant and Antimicrobial Agents in Meat and Meat Products. Molecules. 2023;28(5), 2293.

26. Ukwatta KM, Lawrence JL, Wijayarathna C. The study of antimicrobial, anti-cancer, anti-inflammatory and α-glucosidase inhibitory activities of Nigronapthaphenyl, isolated from an extract of *Nigrospora sphaerica*. Mycology. 2019;10(4):222-228.
27. Hennessy RC, Christiansen L, Olsson S, et al. A broad-host range dual-fluorescence reporter system for gene expression analysis in Gram-negative bacteria. J Microbiol Methods. 2018;144:173-176.
28. Jarolim K, Wolters K, Woelflingseder L, et al. The secondary Fusarium metabolite aurofusarin induces oxidative stress, cytotoxicity and genotoxicity in human colon cells. Toxicol Lett. 2018;284:170-83.