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Production of Antimicrobial Bioactive Compounds from Endophytic Fungi Against *Escherichia coli* Strain NRC1 Isolated from the African Catfish

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ABSTRACT

In the present study, *Escherichia coli* was successfully isolated from a catfish sample collected from the El-Moheet drain in Giza, Egypt, with a prevalence rate of 20.8%. The bacterial isolates were confirmed through biochemical tests, demonstrating characteristics consistent with *E. coli*, such as indole production, nitrate reduction, and acid production. The strain was further confirmed by 16S rRNA sequencing and deposited in GenBank (PQ218973.1). In parallel, five endophytic fungi were extracted from seagrasses in Wadi El-Natron, El-Beheira, Egypt, and exhibited notable antimicrobial properties. Crude extracts from these fungi, particularly the F2 isolate, showed strong antimicrobial activity, with an efficacy of 89.40 ± 2.44% against pathogenic microorganisms. The most potent endophyte was identified as *Aspergillus* sp. through morphological and genetic analysis, classified as *Aspergillus* sp. isolate NRC1, and deposited in GenBank (PQ270262.1). GC-MS analysis of the fungal extracts revealed 36 bioactive compounds, with trans-1,4-diacetoxy-2-butene being the most abundant. ADME analysis of this compound indicated favorable physicochemical properties, including high gastrointestinal absorption and blood-brain barrier permeability. It exhibited no significant inhibitory effects on major cytochrome P450 enzymes, suggesting a low risk of drug interactions. Despite potential challenges in synthetic optimization, trans-1,4-diacetoxy-2-butene shows promise for further therapeutic development.

INTRODUCTION

Discussions on sustainability in aquaculture have gained momentum globally over the last twenty years **(Naylor *et al.*, 2021)**. Currently, aquaculture has emerged as the

most rapidly expanding food industry **(Sudheesh *et al.*, 2012)**. This sector is critical to food safety and security, especially in regions facing food insecurity, while also supporting the livelihoods of millions worldwide **(Irshath *et al.*, 2023)**. The United Nations' Food and Agriculture Organization (FAO) has projected that to meet the requirements of the increasing global populace by 2050, there will need to be a 70% rise in the global food and feed provision **(De Bruijn *et al.*, 2018)**. Aquaculture production currently represents the most rapidly growing sector of animal food production globally. In 2019, the total output of aquaculture reached 85.3 million tons, indicating a 3.7% increase from the previous year. The leading producers in 2019 included China producing 48.2 million tons, followed by India with 7.8 million tons, Indonesia at 6.0 million tons, Vietnam with 4.4 million tons, Bangladesh at 2.5 million tons, and Egypt with 1.6 million tons **(FAO, 2021)**. Within the aquaculture sector, outbreaks of opportunistic pathogens can be triggered by various stress conditions, including poor physicochemical and microbiological water quality, inadequate nutrition, and high densities of stockings. The relationships between the host, infections, and their surroundings frequently have an impact on these variables **(Burge *et al.*, 2014)**. The likelihood of serious disease outbreaks can be greatly increased by altering any of these factors or the climate **(Hegde *et al.*, 2023)**. High concentrations of pollutants and suspended particles may lead to malformations and mortality in both juvenile and adult fish **(Kumari & Teacher, 2020)**. Marine fish, in particular, face numerous environmental challenges, including exposure to toxins and biological threats. These factors can severely suppress the immune responses of marine species in affected environments, resulting in a higher incidence of bacterial infections **(Olafsen, 2001)**.

Intensive and semi-intensive aquaculture systems frequently experience outbreaks of viral, bacterial, parasitical, and fungal infections, resulting in significant production losses and substantial economic impacts **(Moustafa *et al.*, 2014; Radwan *et al.*, 2022; Elgendy *et al.*, 2023a; Elgendy *et al.*, 2023b)**. The financial worth of tilapia summer death in Egypt is valued at over 100 million US dollars **(Fathi *et al.*, 2017)**. In this context, a seven-year field trial held in Alabama, USA, expounded those annual economic consequences due to diseases in the commercial West Alabama catfish aquaculture with 11.1 million US dollars **(Abdelrahman *et al.*, 2023)**. Particularly, disease losses analysis revealed that bacterial diseases were the most common source of the above epizootics, resulting in an 83% loss of production initializer **(Elgendy *et al.*, 2024)**.

Life-threatening viruses and bacteria that are resistant to drugs are proliferating, which has created a growing demand for novel and advantageous chemicals. The conventional reservoirs of natural bioactive substances, encompassing temperate flora and soil microorganisms, have proven ineffective in addressing the global surge in fungal pathogens and chronic ailments, particularly among individuals with compromised immune systems, such as transplant recipients and cancer patients, as a result of the

administration of various immunosuppressive medications **(Jeewon, *et al.*, 2019; El-Bondkly *et al.*, 2020)**. Certain microbiological products have been observed to exhibit distinctive characteristics exclusive to particular biotopes. Consequently, it is advisable to prioritize exploring novel drugs with innovative mechanisms of action by targeting organisms that inhabit unique biotopes. In the present context, microorganisms residing in distinct and uncharted ecological roles, like endophytes, which occupy biotopes encompassing maritime vegetation (such as mangrove plants, algae, seagrass, and driftwood), invertebrates (e.g., sponge, coral, crab, holothurian, and bivalve), vertebrates (mainly fish), and various other minor marine phylum, now appear to be an astonishing reservoir of bioactive pharmaceutical interventions. Fungal endophytes consist of a polyphyletic group mostly composed of ascomycetous fungi that only reside in healthy host tissues during one or more phases during their life cycle without displaying any discernible disease symptom or negatively affecting their host(s) **(Tallei, 2019; Dastogeer *et al.*, 2018)**.

Endophytic fungi can produce a wide range of secondary metabolites with medicinal potential, offering an effective platform for identifying new antibiotics. As previously mentioned, endophytic fungi—regardless of their marine origin—are considered a valuable resource for developing innovative therapeutic compounds. Therefore, investigating endophytic fungi that thrive in marine environments, along with assessing their biological properties, including antibacterial and antitumor effects, is essential **(Al-Rajhi *et al.*, 2024)**.

MATERIALS AND METHODS

1. Sample collection

In the current investigation, samples of the African catfish (*Clarias garipinus*) were obtained on three separate occasions in October 2023 from El-Moheet Drain, Abu-Rawash-Giza, Egypt. On each occasion, 30 catfish were gathered. All fish specimens were gathered randomly and put in individual sterile plastic bags filled with sterile water to clean their surfaces before being transported to the laboratory in insulated containers. Ultimately, a total of 90 samples, specifically the gills from each fish, were subjected to analysis in this study.

2. Fish sample preparation and *E. coli* isolation

To determine *E. coli* presence, each gill sample was subjected to an investigation. The gill samples underwent enrichment in 1:10 dilution of sterile TSB (trypticase soy broth, Himedia). About 5g of the gill tissue from each catfish was soaked in 225mL TSB. After 18 ± 2 hours of heat incubation at 37°C followed, a loopy of the broth culture was streaked for purified cultures. The isolate was subsequently incubated for 18 to 24 hours at 35-37°C temperatures on eosin methylene blue (EMB) agar plates (HiMedia). The *E. coli* isolates were re-streaked on nutrient agar (NA) agar plates (Himedia) to confirm

bacterial isolates' purity. The indole test was conducted on Gram-negative species for biochemical identification.

The isolated *E. coli* strains were stored at -70°C in a 30% glycerol solution in brain heart infusion broth (BHI; Himedia). For each isolation, a 10µL loop of bacterial culture was fixed in 500µL of 100% ethanol in a 1.5mL tube. These tubes were then stored at -20°C for PCR analysis or subsequent DNA extraction. *E. coli* ATCC 25922 served as an effective control during subculture on selected agar media and PCR analysis **(Rheman *et al.*, 2024)**.

3. Standard strain

The reference strain *Escherichia coli* (ATCC25922) was utilized to validate the isolation, identification, and detection of *E. coli* isolates.

4. Sample, isolation, and endophyte purification

The sea grass plant samples were acquired from different sites around the lake Wadi-El natron valley. The plants' stems, roots, and leaves were collected. Plant samples were sliced into small pieces after three washing in aseptic conditions with sanitized seawater, samples were cut into small slices to expose the inner tissue surface. Every sample was suspended in five milliliters of sterile seawater and then maintained for 30 minutes at 30°C in the water bath (Tiny SHD 700, Hasegawa Labotek Engineering, Chiba, Japan). A series of tenfold dilutions were performed by making them in sterile seawater and plating 100µl of an overnight culture on pretreated potato dextrose agar (PDA) plates to get pure colonies of endophytes **(Bovio *et al.*, 2019)**.

5. Fungal preparations

Commercial rice (100 grams) combined with 50% pure seawater (100 milliliters) was added to a solid medium in an Erlenmeyer flask and sterilized at 121°C for 15mins and cooled to ambient temperature. Filtrate (neat broth) at a 50% ratio was mixed with 1X Potato Dextrose Salt (PDS) broth, and afterwards, five endophytic fungus isolates (F1-F5) were separated, plunged in the mixture in aseptic conditions, and then incubated at 30°C for a seven-day period, as mentioned above. After the incubation period, the culture broth of each fungus isolate was extracted two times, with the same quantity of ethyl acetate. The organic extract was mixed together and dried out by evaporation in vaccum before being subjected to biological and antibacterial testing procedures to yield a crude extract.

6. Antimicrobial activity

The antibacterial activity was measured through experiments on polystyrene flat plates with 96 wells altered for the bacterial growth tests. The separate wells received 10µl of extraction test at ultimate concentrations of 500µl/ ml and another 80µl of Lysogeny broth then 10µl of a suspensions of a log phases culture of isolated bacteria to

form a 100µl volume. The prepared plates underwent incubation at 37 Celsius for an overnight period. Clear zones were observed where the tested components had clear antibacterial effects. The tested components were sampled when the growing media in the wells seemed opaque from those without antibacterial effects. The control samples were the untreated pathogens. After 20 hours, the optical density (OD) was assessed at an emission wavelength of 600nm utilizing a SPECTROstar Nano-microplate analyzer (Germany) **(Hamed *et al.*, 2020)**.

7. Identification of most potent fungal isolate using phenotypic study

The fungal isolates were identified based on colony formation pattern, conidial development, and pigmentation, the fungal isolates were recognized by culture and morphological features **(Tafinta *et al.*, 2013)**.

7.1. Genetic confirmation of the potent isolates

To identify the bacterial and fungal isolates exhibiting strong antibacterial activity, molecular identification was performed using the Qiagen DNeasy Kit for DNA extraction, following the experimental manual. For the bacterial isolate, PCR amplification was conducted with the forward primer 5' AGAGTTTGATCMTGGCTCAG 3' and the reverse primer 3' TACGGTACGTACGTTGTTACGACTT 5'. The fungal isolate was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The reaction mixtures contained 1 µg of fungal genomic DNA, 1 µL of 20 µM primers, a 0.2 mM mix of 10 mM dNTPs, two units of Taq DNA polymerase (Applied Biosystems, USA), and 10µL of 5× reaction buffer (containing 15 mM MgCl2).

The PCR temperature profile included an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds, with a final extension step at 72°C for 5 minutes. The PCR byproducts were purified using ThermoFisher Scientific's GeneJET purification kit and sent to Macrogen in South Korea for sequencing. The 16S rRNA gene sequences of the bacteria were subjected to BLAST analysis and alignment in GenBank (NCBI, Maryland). The gene sequences were submitted to the GenBank database, and a phylogenetic tree was constructed using neighbor-joining analysis with the software MEGA10.

8. Purification and structure elucidation of the bioactive compound

The extraction initially occurred using column chromatography in flash. Normal-phase silicon was phase packed into a column with a diameter of 7cm. The crude extract (3.8g) was dropped on the column with a silica gel to crude extracts ratio of 20:1. In all, 110 portions (5ml each of them) were gathered. TLC was used to locate the fractions that

contained compound 1. The fraction with good antibacterial activity was isolated, and structural elucidation was done using GC-Mass spectrometry.

8.1. GC-MS analysis

The GC-MS analysis was conducted using techniques similar to those reported by **Madkour *et al.* (2017)**. A Thermo Trace GC Ultra coupled with an ISQ Only Quadrupole MS, equipped with TG-5MS fused silica capillary columns (30 m, 0.251 mm, 0.1 mm film thickness), was utilized. The instrument featured a particle ionization mechanism with an ionization energy of approximately 70 eV. Helium was used as the carrier gas at a steady flow rate of 1mL/ min.

The injection port temperature was set to 250°C, while the MS transfer line temperature was maintained at 280°C. The oven temperature program began at 50°C (held for 2 minutes) and then increased gradually at a rate of 7°C/min to 150°C. This was followed by a rate of 5°C/min to 270°C (held for 2 minutes), and finally increased to 3.5°C/min to 310°C (held for 10 minutes). Mass scans were recorded from m/z 50 to 650, and the relative peak area of each component was measured.

The chemicals were tentatively identified by analyzing the mass spectra and retention time data in comparison with the NIST and WILLY library statistics of the GC/MS instrument.

9. In silico predictions ADME-related physicochemical properties and toxicity

The obtained compound's ADME linked to physico-chemical features was predicted with the Swiss/ADME web tools **(Daina *et al.*, 2017)**. On the other hand, the ProToxii website was utilized to evaluate in silicon toxicity for substances **(Banerjee *et al.*, 2018)**.

RESULTS AND DISCUSSION

1. Isolation of bacterial isolate from fish sample

Escherichia coli is frequently found in a warm-blood animals' gastrointestinal tracts, and the majority of its several strains do not have any detrimental impact on their hosts' health. While the majority of *E. coli* strains are non-pathogenic to humans, prior studies have shown the presence of virulence factors in certain strains **(Koitabashi *et al.*, 2006)**, such as the Drain of El-Moheet, Abu-Rawash, Giza, Egypt. The *E. coli*-positive samples were among a total of 30 catfish samples collected from the drain. The maximum proportion prevalence of *E. coli* bacteria (20.8%) occurred in the samples. Positive culture isolates were subjected to biochemical tests to confirm *E. coli* isolates. Based on the results of biochemical tests, it was confirmed that Gram-negative bacteria of the strain *Escherichia coli* were capable of breaking indoles, generating acid, and reducing nitrate. **Soku *et al.* (2024)** isolated 114 types of generic *Escherichia coli* were

isolated from related fish species and the channel catfish. In a different investigation, 17 *E. Coli* strains were recovered from water and farmed catfish from four different places in aquaculture within and surrounding Kuching, Sarawak **(Samuel *et al.*, 2011)**.

2. Isolation of endophytic and antimicrobial activity

Fungal endophytic population structure: the process of colonization within the host tissue promotes tolerance to particular environmental conditions that are essential to plants' survival. Comprehensive study on fungal endophytes has shown how important they are for supplying nutrients, fostering growth, controlling both abiotic and biotic stress, and supporting plant development. Researchers may investigate endoporotic fermentation when seeking natural compounds facilitated by endophytes. Extensive evidence supports the production of several bioactive metabolites with possible pharmacological properties by endophytic fungi **(Tiwari, 2015)**. Therefore, five endophytic fungi (F1-F5) were obtained from sea grasses gathered in Wadi El-Natron in Egypt's El-Beheira province. With the use of PDA medium, these fungal organisms were isolated from a variety of plant parts, comprising stems, roots, and leaf surfaces.

The antimicrobial efficacy of the crude extracts derived from five endophytic microorganisms were assessed *in vitro* toward various pathogenic microorganisms, as detailed in Table (1). According to the results of antimicrobial activities, all isolates' extracts showed antimicrobial activities against the tested bacterial isolate. Therefore, the most potent one was F2, giving 89.40 ±2.44%. Fungal endophytes are recognized as significant archives of bioactive compounds with diverse biological properties, including antibacterial, antioxidant, anticancer, and antiviral effects. Numerous bioactive compounds, substances produced by endophytic fungus, such as steroid hormones isocoumarins, quinones, terpenoids, alkaloids, flavonoids, peptides, and phenolics showed notable effectiveness against a variety of pathogenic microbes **(Khalil *et al.*, 2021)**.

Table 1. Antimicrobial activity of crude extracts

	Antimicrobial activity (%)				
Extracts	**F1**	**F2**	**F3**	**F4**	**F5**
Activity	15.88±1.34	89.40±2.44	36.89±1.89	74.12±1.04	20.58±2.09

3. Identification of the most active endophytes

Each isolated endophyte received biological testing for antibacterial ability. Morphological and genetic identification would be performed to identify the most powerful isolate. The colonies had colony of intense green along the border, reversing from yellow to brown, and pale greenish-yellow colonies that grew to a diameter of 5–6cm at 28°C in 7 days on Czapek's medium. The conidiophore diameter of the radiating conidial head was 15.0µm. The vessel sized 29.0µm (± 0.5) in size and had uniseriate or

biseriate sterigmata. The conidia were globose, measuring 5.5µm (± 0.25) and the primary conidia measured 7.8 x 4.5µm (± 0.5).

The F2 isolate's 18S rRNA genes was blasted to the GenBank databases utilizing the BLAST tool (http://www.blast.ncbi.nlm.nih.gov/Blast). The 18S rRNA genes sequence of isolate F2 were surveyed, detected then contrasted with others acknowledged genes in the GeneBank databases. The result indicated that there was 100% homology between the F2 and *Aspergillus* sp. 18S rRNA gene sequences. Using MEGA 10, a phylogenetic tree based on the neighbor-joining approach was built and examined. After being discovered, the strain F2 was categorized as an alternative. Isolate NRC1 *Aspergillus* sp. based on DNA sequence and morphological characteristics. The data were submitted to GenBank, and the strain was deposited as PQ270262.1 (Fig. 1).

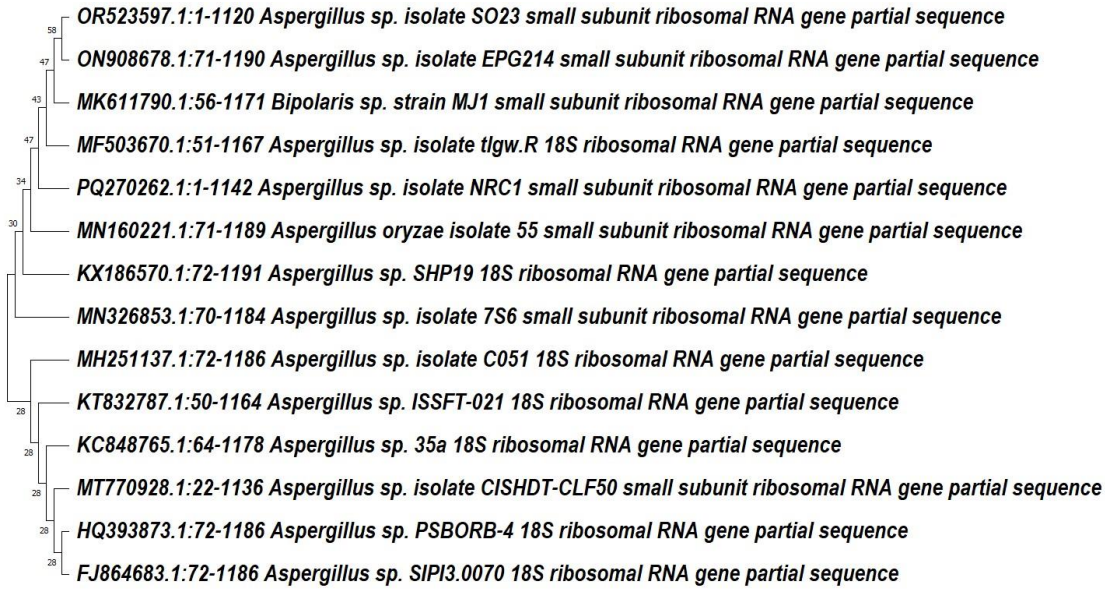

Fig. 1. Constructed phylogenetic tree of *Aspergillus* sp. isolate NRC1

While the bacterial isolate belonged to the *E. coli* genus, it was recognized as *Escherichia coli* strain NRC1 (Accession no. PQ218973.1) when the nucleotide sequence was entered into the GenBank sequence database (Fig. 2).

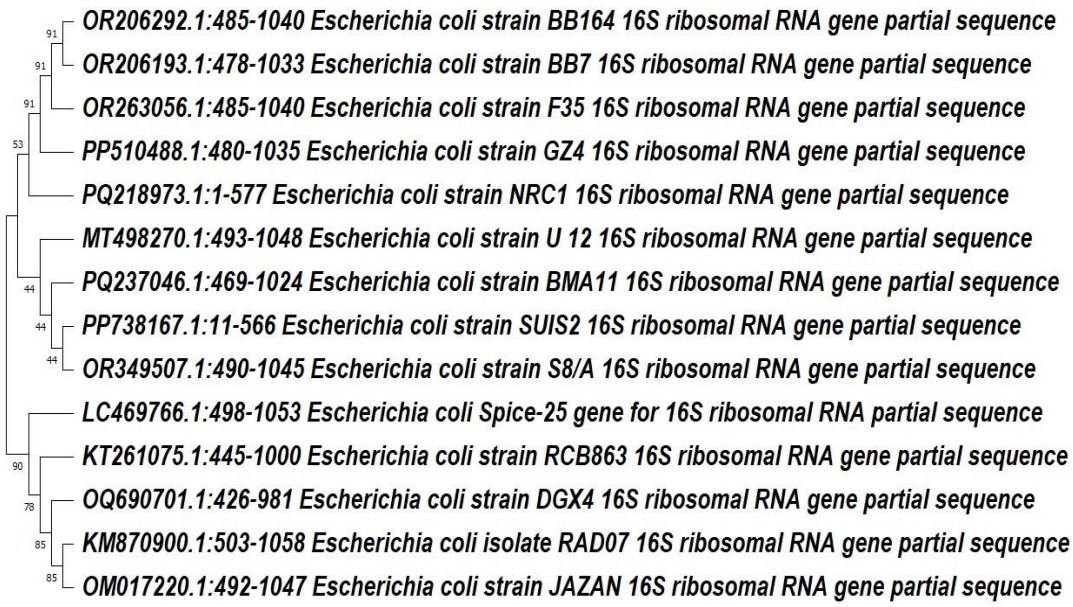

Fig. 2. Constructed phylogenetic tree of *Escherichia coli* strain NRC1

4. GC-MS investigation of the crude extracted from *Aspergillus* sp. isolate NRC1

The extract of crude extracted from *Aspergillus* sp. isolate NRC1 was exposed to GC-MS analysis, and the data obtained yielded a total of 36 compounds. The total amount calculated peak zones of the identified chemicals were 74.54%; the structural possibilities of the identified compounds are shown in Table (2). Key compounds were detected such as 5-butyl-1,2,4-trioxalane constituting 10.67%, trans-1,4-diacetoxy-2-butene constituting 9.68%, and 3,5-diphenyl-3,5-(9,10-phenanthylene)tricyclo[5.2.1.0]decane-4-one-8-exo-9-endodicarboxylic acid diacetoxymethylester constituting 3.18%. The calculated total peak area by integrating the peak areas of the key compounds was 23.53%. The Preparative TLC analysis of the fractions of the petroleum ether extract unveils the isolated compounds. Identification was performed using computer-based searches of user-referenced libraries, matching the acquired mass spectra with standard spectra **(Madkour *et al.*, 2017; Abdel-Wareth *et al.*, 2019; Shawky *et al.*, 2019; Khalaf *et al*., 2021)**.

Table 2. Chemical compositions of crude extracted from *Aspergillus* sp. isolate NRC1

No.	R_t	Area %	M.W.	M.F.	Identified compounds
1	5.14	9.68	172	$C_8H_{12}O_4$	*trans*-1,4-Diacetoxy-2-butene
2	5.22	10.67	132	$C_6H_{12}O_3$	5-Butyl-1,2,4-trioxalane
3	5.45	1.20	204	$C_8H_{16}N_2O_2S$	Bis(1-pyrrolidinyl)sulphone
4	5.81	1.90	174	$C_5H_3BrO_2$	5-(Bromomethylene)-2(5H)-furanone
5	5.98	2.75	64	$C_2H_2F_2$	Ethene, 1,1-difluoro
6	6.27	1.87	642	$C_{37}H_{46}N_4O_6$	2,8-Bis(2-acetoxyethyl)-7,13-dimethyl-3,8,12,17-tetraethyl-1,19,21,24-tetrahydro-1,9-bilindione
7	7.62	1.39	488	$C_{30}H_{48}O_5$	Sodwanone O
8	8.32	2.07	198	$C_{13}H_{26}O$	4-(2'-Methylprop-1'-yl)-2-nonanone
9	8.85	1.83	692	$C_{44}H_{44}N_4O_4$	N,N'-Dicyclohexyl-1,7-dipyrrolidinylperylene-3,4:9,10-tetracarboxylic acid bisimide
10	8.98	1.48	530	$C_{36}H_{26}N_4O$	2-[4-(5-Oxo-4,4-diphenyl-4,5-dihydro-1-himidazol-2-yl)phenyl]-4,5-diphenyl-1-himidazole
11	13.02	1.22	191	$C_{12}H_{17}NO$	N-acetyl-4-methylamphetamine
12	15.36	1.39	732	$C_{42}H_{44}N_4O_8$	13-(Ethylidenedioxy)cyclohexadieno[1,6-b]phylloerythrin trimethyl ester
13	17.85	1.45	636	$C_{43}H_{48}N_4O$	meso-(2-Formylvinyl)-1,2:3,4:5,6:7,8-tetrapentanoporphyrin
14	20.05	1.94	276	$C_16H_{11}F_3O$	1-Trifluoromethyl-3-phenylinden-1-ol
15	21.06	1.66	682	$C_{38}H_{68}Br_2$	2,5-Dibromo-1,4-dinhexadecylbenzene
16	22.35	1.81	635	$C_{18}H_{10}Br_5N$	(4-Bromophenyl)bis(-2,4-dibromophenyl)amine
17	23.84	1.33	357	$C_{21}H_{31}N_3O_2$	1,3-Dimethyluracilo[11](2,4)pyridinophane[5,7-undecamethylene-1,3,6-trimethylpyrido[2,3-d]pyrimidine-2,4-(1H,3H)-dione]
18	27.54	1.25	689	$C_{45}H_{31}N_5O_3$	2-Methoxy-3-nitro-5,10,15,20-tetraphenyl-2,3-dihydroporphyrin
19	27.84	1.58	266	$C_{18}H_{18}O_2$	(3R*,4R*)-3,4-diphenyl-2,5-hexanedione
20	29.82	1.75	686	$C_{41}H_{66}O_8$	(2R)-8,13-epoxy2,2-(8',13'-epoxy-2'-bmethoxy-3'-oxolabdane-1'a,2'a-diyldioxy)-1a-hydroxylabdan-3-one
21	30.37	1.74	696	$C_{46}H_{52}N_2O_4$	2-[2,6-Bis(hex-5-enoxy)phenyl]-9-[2,6-bis(pent-4-enoxy)phenyl]-1,10-phenanthroline
22	30.59	1.61	678	$C_{45}H_{58}O_5$	7,13,19,25-Tetratertbutyl-27,28,29,30-tetrahydroxy-2,3-bishomo-3-

					oxacalix[4]arene
23	31.21	1.30	596	$C_{40}H_{32}N_6$	m-Bis(3,4-diamino-2,5-diphenyl-6-pyridino)benzene
24	31.55	3.18	708	$C_{44}H_{36}O_9$	3,5-Diphenyl-3,5-(9,10-phenanthylene)tricyclo[5.2.1.0] decane-4-one-8-exo-9-endodicarboxylic acid diacetoxymethylester
25	31.98	1.35	694	$C_{48}H_{62}N_4$	2,7,12,17-Tetraethyl-3,5:8,10:13,15:18,20-tetrakis(2,2-dimethylpropano)porphyrin
26	33.43	1.66	704	$C_{43}H_{44}O_9$	Dimethyl-5"-(1,1-Dimethylethyl)-2,2',2",2"',2""-penta methoxy[1,1':3',1":3",1"':3"'.1""-quinquephenyl]-3,3""-dicarboxylate
27	34.09	2.67	596	$C_{17}H_{13}Cl_9O_4$	Monohydrokelevan
28	35.55	2.29	752	$C_{35}H_{64}N_4O_8Si_3$	2-bis(ethoxycarbonyl)methyl-9-(2,3,5-tri-O-(2-methylprop-2-yl)dimethylsilyloxy-á-D-ribofuranosyl)purine
29	36.35	1.23	205	$C_8H_3N_3O_2S$	2-Cyano-6-nitrothiazole
30	36.62	1.33	682	$C_{35}H_{37}Cl_3N_4O_4$	ë-chloro-2,4-bis(2-chloroethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]1,3,5-trimethylporphyrin
31	40.12	1.33	638	$C_{12}H_4Br_6O$	2,2',3,3'.4,4'-Hexabromodiphenyl ether
32	40.23	1.26	352	$C_{14}H_{16}N_4O_5S$	Diethyl[(3,4-dihydro-4-oxo-2-quinazolinyl)thio]bicarbamate
33	40.51	1.69	632	$C_{25}H_{16}Br_4$	4,4',4",4"'-Tetrabromotetraphenylmethane
34	40.85	1.60	188	$C_{13}H_{16}O$	(E)-5-Methyl-1-phenylhex-2-en-1-one
35	41.63	1.43	671	$C_{29}H_{39}Br_2NO_7$	1-Acetyl-4,4-bis[4-(3-bromopropoxy)-3,5-dimethoxyphenyl]piperidine
36	43.13	1.40	744	$C_9H_4Br_8$	5-(Dibromomethyl)-1,3-bis(tribromomethyl)benzene
		T% 74.54			

5. ADME physiochemical studies of the most abundant compound

The compound *trans-1,4-Diacetoxy-2-butene* (C8H12O4) exhibits favorable physicochemical and pharmacokinetic properties that are critical for its potential as a bioactive molecule. With the molecular weight of 172.18g/ mol, it satisfies Lipinski's rule of five, showing no violations, which suggests excellent oral bioavailability. Its total polar surface area (TPSA) of 52.60 Å², in conjunction with a consensus log po/w value of 1.04, indicates a moderate balance between hydrophilicity and lipophilicity. This balance is essential for passive membrane permeability, which is supported by the compound's high gastrointestinal (GI) absorption, as shown in Fig. (3) (Bioavailability Radar Chart). Water solubility analysis reveals high solubility, with values ranging from 17.7 to 33.3mg/ mL across different predictive models. These results suggest good aqueous

solubility, enhancing its absorption potential in biological environments. The compound is permeable across the blood-brain barrier (BBB), as shown in the boiled egg chart (Fig. 4), highlighting its capacity of central nervous system (CNS) activation. Furthermore, it isn't a substrate for P-glycoprotein (P-gp), reducing the likelihood of active efflux, which enhances its brain penetration efficiency.

The absence of inhibitory effects on major cytochrome P450 (CYP) enzymes, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6 as well as CYP3A4, underscores its low likelihood of metabolic interactions and suggests a favorable pharmacokinetic profile with minimal risk of drug-drug interactions. The compound's skin permeation rate is relatively low (Log Kp = -7.12 cm/s), which may limit its use in transdermal formulations but does not impede its oral or intravenous administration routes. Despite its bioavailability score of 0.55, a lead-like molecule, it presents challenges in synthetic accessibility due to the presence of isolated alkenes and multiple ester groups. These medicinal chemistry alerts warrant careful optimization in future analog design for therapeutic applications.

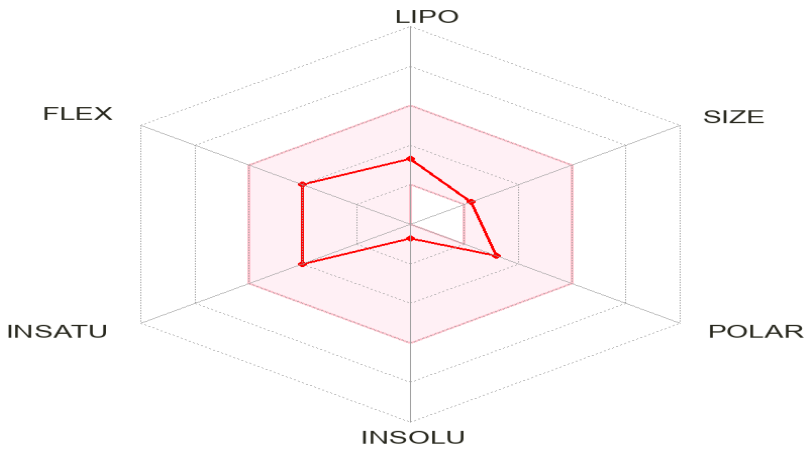

Fig. 3. Bioavailability radar chart

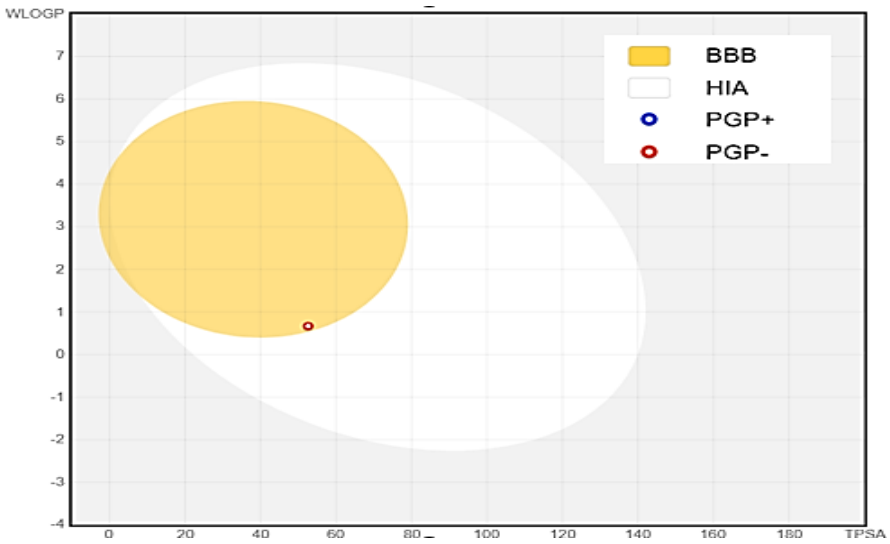

Fig. 4. Boiled egg chart

CONCLUSION

In summary, this study successfully isolated *Escherichia coli* from the catfish, highlighting microbial contamination in the aquatic environment and its implications for water quality and public health. Additionally, five endophytic fungi from seagrasses were isolated, with *Aspergillus* sp. (F2 strain) demonstrating significant antimicrobial activity. GC-MS analysis revealed trans-1,4-Diacetoxy-2-butene as a key bioactive compound with promising pharmacokinetic properties, supporting its potential in drug development. However, challenges related to the compound's synthetic accessibility and medicinal chemistry optimization must be addressed for clinical viability. Future research should focus on improving synthesis, conducting *in vivo* studies, and exploring the compound's efficacy against antibiotic-resistant pathogens. The findings underscore the potential of marine ecosystems as sources of new therapeutic agents, particularly in combating antimicrobial resistance.

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