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Bioactive Compounds of *Aspergillus niger*: A GC-MS and in Silico Investigation

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ABSTRACT

Background *Aspergillus niger*, a ubiquitous fungus, is well known for producing diverse secondary metabolites with potential applications in medicine, agriculture, and industry. These metabolites often play crucial roles in microbial interactions and hold promise for therapeutic development.
Aim The study aimed to isolate, characterize, and identify secondary metabolites of *Aspergillus niger* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, and to evaluate their antimicrobial efficacy along with in silico predictions of their biological relevance and therapeutic potential.
Methodology Metabolites were extracted from *A. niger* cultures grown under optimized conditions. GC-MS analysis identified 30 compounds, of which 16 major peaks were selected for detailed study. A total of 26 bioactive compounds were screened, followed by ADME analysis to assess pharmacological suitability. Antimicrobial assays were performed against *Escherichia coli* and *Bacillus subtilis*. In silico bioinformatics approaches included Gene Ontology (GO) analysis, target prediction using Target Net, pathway enrichment studies, disease association mapping, and protein-protein interaction (PPI) network construction.
Results Among the screened metabolites, Dichloroacetic acid and 1-Dodecanol qualified the ADME analysis criteria. Antimicrobial testing revealed variable activity, with 50 µg/ml concentration showing a larger zone of inhibition against *Bacillus subtilis*. GO analysis highlighted the metabolites' involvement in various biological processes, molecular functions, and cellular activities. Target prediction and pathway enrichment revealed their interaction with specific proteins and metabolic pathways, while disease association studies suggested therapeutic applications. PPI network analysis further identified critical nodes and hubs targeted by the metabolites.
Conclusion This study provides a comprehensive insight into the secondary metabolites of *Aspergillus niger*. By integrating GC–MS chemical profiling, antimicrobial assays, and advanced bioinformatics analyses, it highlights the potential of these metabolites as antimicrobial agents and their promising role in drug discovery and therapeutic development.

Keywords: *Aspergillus niger*, Secondary metabolites, GC-MS analysis, In silico studies, antimicrobial activity

INTRODUCTION

Fungi, particularly species belonging to the *Aspergillus* genus, are well known for their ability to produce a wide variety of bioactive secondary metabolites, some of which have demonstrated strong antimicrobial, antifungal, and anticancer properties (Niazi et al., 2023). *Aspergillus niger*, a filamentous fungus, has potential as a source of novel antimicrobial compounds (Yu et al., 2021). It is a ubiquitous organism found in diverse ecological niches, including soil, decaying organic matter, and plant rhizospheres (Mohamed et al., 2022). In the context of biotechnological applications, *A. niger* is widely utilized in enzyme production, organic acid fermentation, and bioremediation (Wei et al., 2022).

The ability of *A. niger* to synthesize various secondary metabolites, such as polyketides, alkaloids, and cyclodepsipeptides, contributes significantly to its bioactive profile (Elfita et al., 2012). *A. niger* has been shown to possess antibacterial activity against a range of pathogens, including *Bacillus subtilis* and *Escherichia coli*, which are frequently used as model organisms in antimicrobial assays (Abdelgawad et al., 2022). However, the full extent of the antimicrobial potential of *A. niger* remains underexplored, especially in terms of its bioactive compound profile (Rodrigo et al., 2021; Bai et al., 2023).

Aspergillus niger is known to produce various secondary metabolites with strong antibacterial properties. These compounds, such as polyketides, alkaloids, and peptides, can interfere with important bacterial functions like cell wall formation, protein synthesis, and membrane stability (Al-Shaibani et al., 2013; Wei et al., 2022). Some of the identified metabolites, like tensyuic acid and nigerazine B, have shown effective activity against harmful bacteria. The antimicrobial action is believed to occur by damaging bacterial cells or blocking vital enzymes needed for their survival (Hameed et al., 2015; Ortega et al., 2021). This makes *A. niger* a promising source for developing new antibiotics, especially against drug-resistant strains (Niazi et al., 2023).

In this study, antimicrobial compounds were extracted from *Aspergillus niger* using methanol, followed by identification through Gas Chromatography-Mass Spectrometry (GC-MS), and tested for activity against *Bacillus subtilis* and *Escherichia coli* (Siddiquee et al., 2012). GC-MS, a robust technique for profiling volatile and semi-volatile compounds, enabled the detection of key metabolites likely responsible for antimicrobial activity (Ortega et al., 2021).

Although prior research has noted the antibacterial potential of *A. niger*, the specific bioactive compounds and their mechanisms remain underexplored (Hameed et al., 2015). After compound identification, in silico analyses assessed drug-likeness based on Lipinski's rule of five to evaluate pharmacokinetic suitability (Ramírez-Rendon et al., 2022). Target prediction was also conducted to model potential interactions between the compounds and bacterial proteins.

Further, gene ontology (GO) analysis provided insight into the biological functions and molecular pathways influenced by the compounds, such as enzymatic activity and cellular regulation (Gerke et al., 2014). Pathway mapping revealed how these metabolites may interfere with critical bacterial processes like protein synthesis and cell wall formation, indicating potential bactericidal mechanisms (Gerke et al., 2014). Lastly, disease association studies suggested possible relevance to human health, broadening the therapeutic scope of these metabolites (Ramírez-Rendon et al., 2022).

The incorporation of such computational studies significantly enhances the drug discovery process by enabling the identification of compounds with multifaceted activities that can target both bacterial pathogens and related diseases. In conclusion, by combining experimental techniques like GC-MS with advanced computational methods, we aim to identify

and characterize potential antimicrobial agents that can serve as the foundation for new therapeutic drugs. This study highlights the importance of integrating modern analytical and computational approaches to accelerate drug discovery, ultimately contributing to the development of safe and effective treatments for bacterial infections, including those caused by resistant strains of *B. subtilis* and *E. coli*.

MATERIALS & METHODS

ISOLATION OF *Aspergillus niger:* *A. niger* was isolated from an onion with black Mold infections using serial dilution method. The infected part of the onion was cut out carefully and crushed with the help of mortar and pestle by adding a drop of distilled water. The inoculum was serially diluted. 150ml of Potato Dextrose Agar (PDA) was prepared and poured into petri plates. The aliquot of diluted inoculum was spread on PDA media, and plates were incubated for 3 -7 days at 25 C. *Niazi, S. K., et al. (2023)*

IDENTIFICATION: Staining was employed to identify and characterize the organism. The pure colonies of fungal isolates were morphologically identified by observing color, mycelia, pigment production, and sporulation. In addition, the morphological, mycelia and spore structures were observed using a compound microscope. *Wei et al. (2022).*

CHARACTERIZATION: The morphological and microscopic characterization were tabulated in the results.

ANTIMICROBIAL ACTIVITY: The preparation of bacterial cultures began with subculturing *Bacillus subtilis* and *Escherichia coli* from their respective stock cultures onto fresh nutrient agar plates, which were incubated at 37°C for 24 hours. A single colony from each culture was then inoculated into 10 mL of sterile nutrient broth and incubated at 37°C with shaking at 120 rpm until the bacterial concentration reached approximately 10^8 CFU/mL. For the preparation of test samples, bioactive compound extracts were dissolved in an appropriate solvent, such as methanol, to achieve the desired concentration. The agar well diffusion method involved preparing Mueller-Hinton Agar (MHA) (Himedia) plates by pouring the medium into sterile Petri plates and allowing it to solidify. The surface of each MHA plate was swabbed with a sterile swab dipped in the standardized bacterial inoculum to create a uniform lawn of bacteria. Wells of 6 mm diameter were punched into the agar using a sterile cork borer, and the agar plugs were removed. Into each well, 50 µL of the bioactive compound solution was added. Positive controls Ciprofloxacin, with standard antibiotic solutions, and a solvent control, with the solvent used to dissolve the bioactive compounds, were also included in separate wells. The plates were then allowed to stand at room temperature for 1 hour to permit diffusion of the compounds into the agar before being incubated at 37°C for 24 hours in incubator. After incubation, the diameter of the inhibition zones around each well was measured using a ruler, and the results were recorded in millimetres (mm) for each test compound and control. *Wei et al. (2022), Kumari et al. (2019), and Hameed et al. (2015).*

GC-MS ANALYSIS: The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df). The components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 220°C during the chromatographic run. 1µL of extract sample was injected into the GC-MS instrument (Gas chromatograph coupled with mass spectrometer), the oven temperature was as follows: 50 °C (2 min); followed by 150 °C at the rate of 15 °C min−1; and 150 °C, where it was held for 2min and then followed by 250°C at the rate of 30°C

min−1. It was held for 8.00 min. The mass detector conditions were: Inlet line temperature 250 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments were from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2014) library. *Siddiquee et al., 2012; Kumari et al., 2019; Ibrahim et al., 2023*.

ADME ANALYSIS: Swiss ADME (https://www.swissadme.ch/) website was opened using a web browser. The structure of the bioactive compounds in the supported format, i.e., Canonical SMILES, was prepared and entered directly into the input box on the Swiss ADME website. The "Run" button was clicked to initiate the analysis. The Swiss ADME website processed the compound's SMILES and generated various pharmacokinetic properties. A detailed report on each compound was presented, which was used to review the results. *Daina et al., 2017; Niazi et al., 2023; Singab et al., 2023.*

TARGET PREDICTION: The TargetNet web server (http://targetnet.csiro.au/) was accessed. On the TargetNet web server, the section for input data submission was located. The Canonical SMILES was pasted into the "SMILES" section. The "Submit" button (or its equivalent) was clicked to initiate the prediction process. The results were tabulated and used for further proceedings. *(Singab et al., 2023; Abdelgawad et al., 2022).*

PROTEIN-PROTEIN INTERACTION NETWORK: The STRING web server (https://string-db.org/) was used. The multiple protein section of the server was selected. The names of all the protein targets or the UNIPROT IDs of the predicted proteins were entered in the search bar. "Homo sapiens" was selected in the Organisms section. The "Continue" option that appeared on the next page was clicked. The Protein-Protein Interaction Network (PPIN) of the predicted targets was displayed, and the results were obtained. The interaction networks, summary tables, and any visualizations generated from STRING were saved or exported. These outputs were used for further analysis or reporting. *(Chaudhary et al., 2019; Niazi et al., 2023; Abdelgawad et al., 2022).*

GENE ONTOLOGY: The Shiny GO 0.76 web server (http://bioinformatics.sdstate.edu/go/) was used. The names or IDs of the proteins of the bioactive compounds were entered into the provided input field. The organism context was specified as *Homo sapiens* (as the best match). The GO category- Biological Process, Molecular Function, or Cellular Component- was selected. The analysis was initiated to identify enriched GO terms associated with the proteins influenced by the bioactive compounds. *(Chaudhary et al., 2019; Niazi et al., 2023; Abdelgawad et al., 2022).*

PATHWAY ENRICHMENT ANALYSIS: The Shiny GO 0.76 web server (http://bioinformatics.sdstate.edu/go/) was accessed. The names or IDs of the proteins of the bioactive compounds were entered into the provided input field. The organism context was specified as *Homo sapiens* (as the best match). The KEGG pathway database was selected. The analysis was conducted to identify enriched KEGG pathways relevant to the bioactive compounds. *(Chaudhary et al., 2019; Niazi et al., 2023; Abdelgawad et al., 2022).*

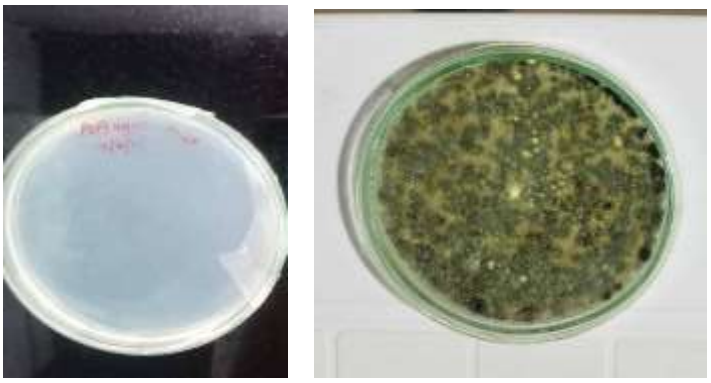
DISEASE ASSOCIATION: The Shiny GO 0.76 web server (http://bioinformatics.sdstate.edu/go/) was accessed. The names or IDs of the proteins of the bioactive compounds were entered into the provided input field. The organism context was specified as *Homo sapiens* (as the best match). Disease-related databases, such as Disease Alliance available in Shiny GO 0.76, were used. The analysis was

initiated to identify diseases or disorders enriched among proteins affected by the bioactive compounds. *(Chaudhary et al., 2019; Niazi et al., 2023; Abdelgawad et al., 2022).*

RESULT

Isolation of sample

Aspergillus culture was isolated from onion samples. Potato Dextrose Agar (PDA) was used for isolation. After 5 days, growth of *Aspergillus* was observed.



(a) *Aspergillus* plates

Fig.1: Isolation of Sample organism

Identification and characterization of sample organism

MACROSCOPIC	CHARACTERISTICS
Initial Appearance	White colonies (early growth)
Colour Change	Dark brownish due to conidia
Colony Edges	Pale yellow edges, Radial fissures
Texture	Powdery texture, Black velvety
MICROSCOPIC	CHARACTERISTICS
Hyphae	Long, thread-like structures, septate
Globular Vesicles	Rounded structures at conidiophore tips
Conidiophores	Long, unbranched, smooth
Conidia	Black, asexual spores
Phialides	Biseriate (two series)

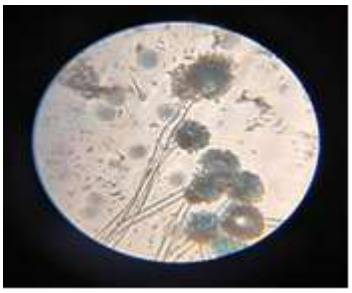


Fig 2: Microscopic observation of *Aspergillus niger*

Antimicrobial activity of sample against other bacteria

The antibacterial activity of bioactive compounds extracted from *Aspergillus niger* was evaluated against *Bacillus subtilis* and *Escherichia coli* using the well diffusion method. Negative Control was water. Positive control was Ciprofloxacin. S1 and S2 was sample 1 and 2 at 100µg/ML and 50µg/ML concentration respectively. Antimicrobial activity was shown majorly on S1 against *E. coli.*

Table 1: Antimicrobial activity of *Aspergillus* extract on *B. Subtilis, E. coli*

Zone of inhibition (mm)		
Culture condition	***B. subtilis***	***E. coli***
Control	0	0
Ciprofloxacin-5ug	14	13
S1(100µg/ML)	2.3	3.4
S2(50µg/ML)	1.9	2.0

(a)Bacillus subtilis (b) E.coli

Fig 3: Anti-bacterial activity

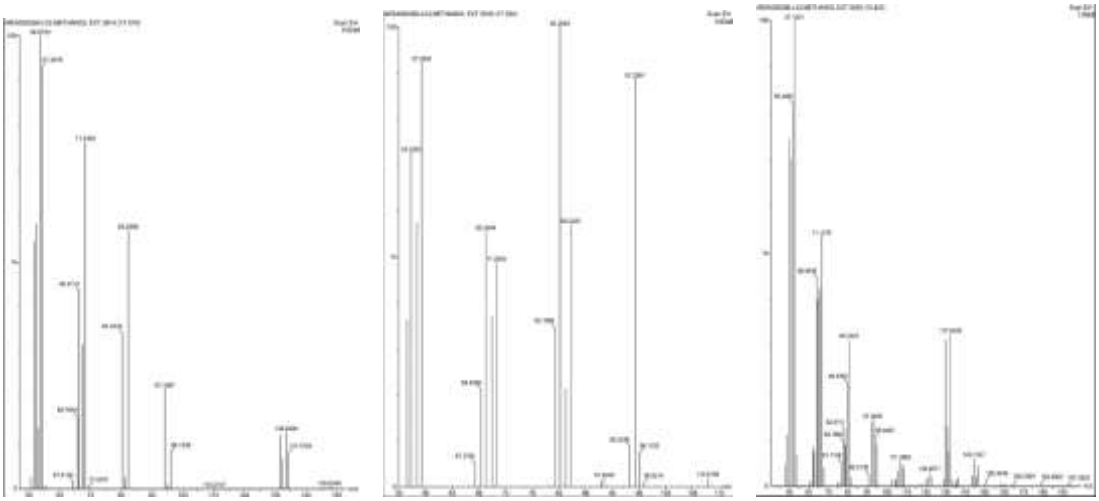
GC-MS analysis of secondary metabolites from *Aspergillus niger*

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on the methanolic extract of *Aspergillus niger* to identify bioactive compounds. The GC-MS chromatogram revealed thirty peaks, indicating the presence of multiple compounds.

The identified compounds are tabulated below in Table 2.

Oxalic Acid Esters: • Oxalic acid, allyl pentadecyl ester • Oxalic acid, allyl hexadecyl ester • Oxalic acid, allyl dodecyl ester • Oxalic acid, allyl undecyl ester • Oxalic acid, allyl octadecyl ester • Oxalic acid, allyl nonyl ester • Oxalic acid, allyl decyl ester	Halogenated Compounds: • Dichloroacetic acid, nonyl ester • Acetic acid, trichloro-nonyl ester • Octadecane, 1-bromo • Hexadecane, 1-bromo • Pentadecane, 1-bromo • 2-Bromo dodecane • Heptadecane, 1-bromo • Undecane, 1-bromo • Tetradecane, 1-bromo • Decane, 1-bromo • Dodecane, 1-bromo • Tridecane, 1-bromo • 1-Bromodocosane
Sulfurous and Nitric Acid Esters: • Sulfurous acid, decyl 2-propyl ester • Nitric acid, nonyl ester • Sulfurous acid, 2-propyl undecyl ester	Alcohols and Alkanes: • Cetene • 1-Dodecanol • 1-Heptadecene

Table 2: Compounds identified from GC-MS analysis of the methanolic extract of *A. niger*



Mass spectrum of secondary metabolites at Retention Time (RT)= 17.0, 17.5, 18.4 respectively

ADME analysis of metabolites identified by GC-MS analysis

To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biological events to occur. Drug development involves the assessment of absorption, distribution, metabolism, and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited (Ramírez-Rendon et al., 2022; Chaudhary et al., 2019).

Physico-chemical properties of the compounds

Six physicochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation.

Drug likeness properties of the compounds

The *"drug-likeness"* assesses qualitatively the chance for a molecule to become an oral drug with respect to bioavailability.

Target prediction of DCA and 1-dodecanol using Target Net

Target prediction for Dichloroacetic acid

The structure-based prediction for Dichloroacetic acid was performed using the Target Net web server. This analysis identified the following potential targets for the compound, providing a comprehensive list of proteins that might interact with these bioactive molecules (Table 3).

Target prediction for 1- Dodecanol

The structure-based prediction for 1-Dodecanol was performed using the Target Net web server. This analysis identified the following potential targets for the compound, providing a comprehensive list of proteins that might interact with these bioactive molecules (Table 3).

COMPOUND	TARGETS
Dichloroacetic acid	Carbonic anhydrase 5A, mitochondrial Beta-lactamase , Carbonic anhydrase 12, Carbonic anhydrase 14 , Nitric oxide synthase, inducible Nitric oxide synthase, endothelial Prostaglandin G/H synthase 1, Carbonic anhydrase 9 ,Macrophage migration inhibitory factor, Polyphenol oxidase 2 ,Glutamate carboxypeptidase 2, Liver carboxylesterase 1, DNA dC->dU-editing enzyme APOBEC-3A ,Muscarinic acetylcholine receptor M4, Muscarinic acetylcholine receptor M1, Sphingosine 1- phosphate receptor 2, Cocaine esterase ,Bcl-2-related protein A1 ,Carbonic anhydrase 7 G-Protein coupled receptor, 35 Farnesyl pyrophosphate synthase ,Glutamate receptor ionotrophic NMDA 1 , Monoglyceride lipase Receptor-interacting serine/threonine-PK2 Aldose reductase ,M-phase inducer phosphatase 2 ,Carbonic anhydrase 5B, mitochondrial ,Carbonic anhydrase 4, Carbonic anhydrase 6 Alkaline phosphatase, tissue-nonspecific isozyme
1- Dodecanol	Fatty-acid amide hydrolase 1 ,Cocaine esterase, Polyphenol oxidase 2 ,Carbonic anhydrase 12 ,Sterol O-acyltransferase 1, Sphingosine 1-phosphate receptor 2, Sphingosine 1-phosphate receptor 4, Acetylcholinesterase, Cytochrome P450 1A2 ,Muscarinic acetylcholine receptor M4, Muscarinic acetylcholine receptor M1, Arachidonate 5-lipoxygenase, Farnesyl pyrophosphate synthase, Cannabinoid receptor 1 ,Carbonic anhydrase 4 ,Liver carboxylesterase 1 ,Carbonic anhydrase 6, 5-hydroxytryptamine receptor 1E ,Nitric oxide synthase, endothelial ,Nitric oxide synthase, brain Nitric oxide synthase, inducible ,M-phase inducer phosphatase 2 , DNA dC->dU-editing enzyme APOBEC-3A Cannabinoid receptor 2, Carbonic anhydrase 5A, mitochondrial ,Carbonic anhydrase 7, Cannabinoid receptor 2, Histone deacetylase 4 ,Casein kinase II subunit alpha, Metabotropic glutamate receptor 2 ,Bcl-2-related protein A1 ,Monoglyceride lipase Sphingosine 1-phosphate receptor 5, Toll-like receptor 9 ,Cannabinoid receptor 2 , Carbonic anhydrase 14, Carbonic anhydrase 5B, mitochondrial

Table 3: Target prediction of DCA and 1-dodecanol using Target Net

Protein-Protein interaction network of DCA and 1-Dodecanol

The protein-protein interaction (PPI) network analysis provides insights into the complex web of interactions that proteins engage in within a cell. In our study, A total of 83 proteins was queried for STRING PPIN analysis, which were mapped against the human proteome. Of which 61 and 62 proteins were presented and connected in the PPIN of Dichloroacetic acid and 1-Dodecanol respectively.

PPIN OF TARGETS OF DICHLOROACETIC ACID

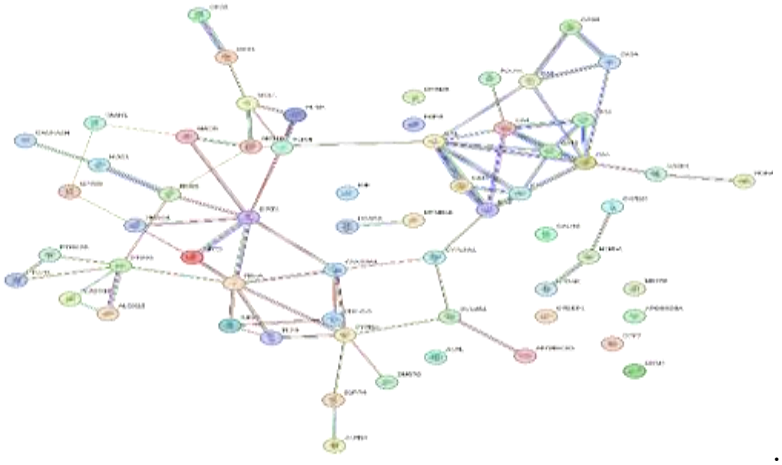


Fig 4: PPIN of Dichloroacetic acid

Network statistics for DCA

Number of nodes	183
Number of edges	1039
Average node degree	11.4
avg. local clustering coefficient	0.485
Expected number of edges	307

PPIN OF TARGETS OF 1 – DODECANOL

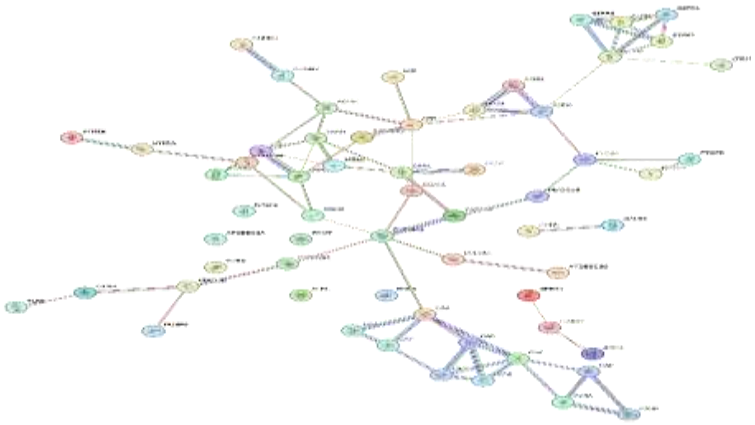


Fig 5: PPIN of 1 – Dodecanol

Network statistics for 1-Dodecanol:

Number of nodes	62
Number of edges	81
Average node degree	2.61
avg. local clustering coefficient	0.495
Expected number of edges	17

Gene ontology of DCA and 1-Dodecanol for biological, cellular and molecular processes

The following Gene ontology functional enrichment analysis was performed for the targets identified in PPIN.

Biological process - In the context of Gene Ontology (GO), a biological process refers

to a series of events or molecular functions with a defined beginning and end, pertaining to the functioning of integrated living units: cells, tissues, organs, and organisms.

DICHLOROACETIC ACID

Fig 6: Gene Ontology of Biology processes for Dichloroacetic acid

1-DODECANOL

Fig 7: Gene Ontology of Biology processes for 1 – Dodecanol

Cellular processes - In Gene Ontology, the cellular component refers to the specific locations within a cell or its extracellular environment where a gene product is active. This could include various cellular structures such as the nucleus, mitochondrion, or plasma membrane.

DICHLOROACETIC ACID

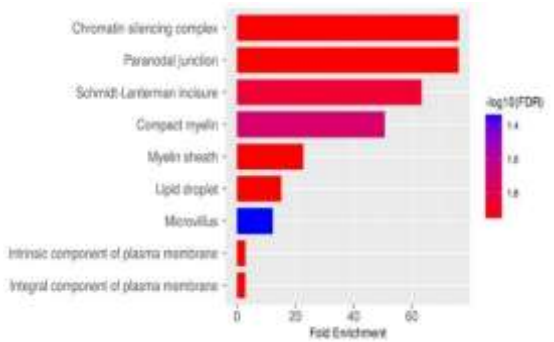


Fig 8: Gene Ontology of Cellular component for Dichloroacetic acid

1-DODECANOL

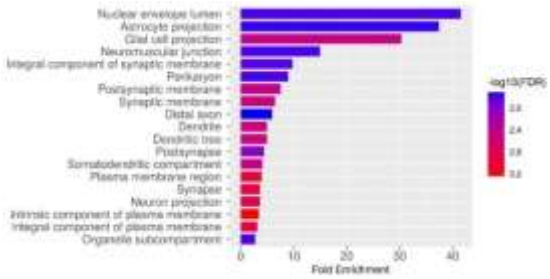


Fig 9: Gene Ontology of Cellular component for 1 – Dodecanol

Molecular function - Molecular function in Gene Ontology describes the elemental activities of a gene product at the molecular level. This can include activities like DNA binding, enzyme activity, or receptor activity which directly relate to the specific biochemical interaction that a protein can perform.

DICHLOROACETIC ACID

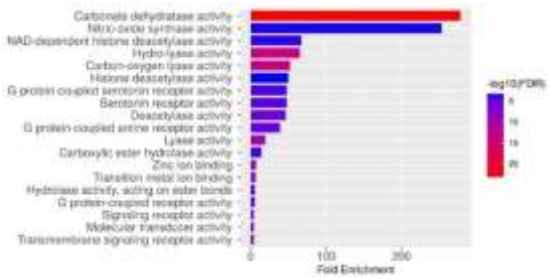


Fig 10: Gene Ontology of Molecular function for Dichloroacetic acid

1-DODECANOL

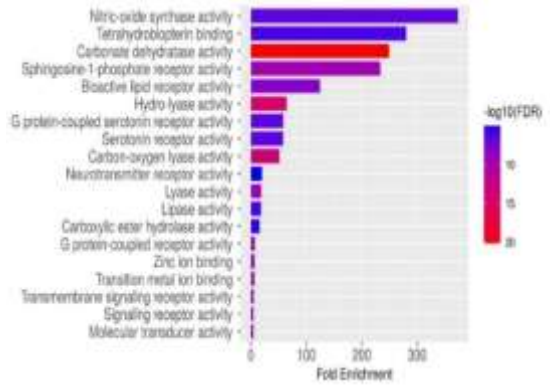


Fig 11: Gene Ontology of Molecular function for 1 – Dodecanol

Pathway enrichment analysis of DCA and 1-Dodecanol showing impact on nitrogen pathway

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis is a powerful tool used to map genes or proteins to known biological pathways. This analysis helps in understanding the roles of these genes or proteins in specific biochemical reactions and processes (Chaudhary et al., 2019).

DICHLOROACETIC ACID

The KEGG pathway enrichment analysis for Dichloroacetic acid identified several key pathways involving the target proteins.

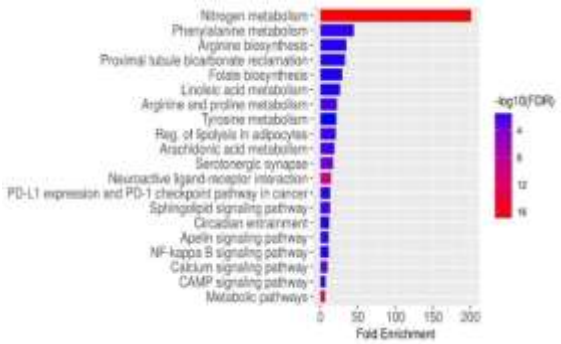


Fig 12: KEGG pathway for Dichloroacetic Acid predicted targets

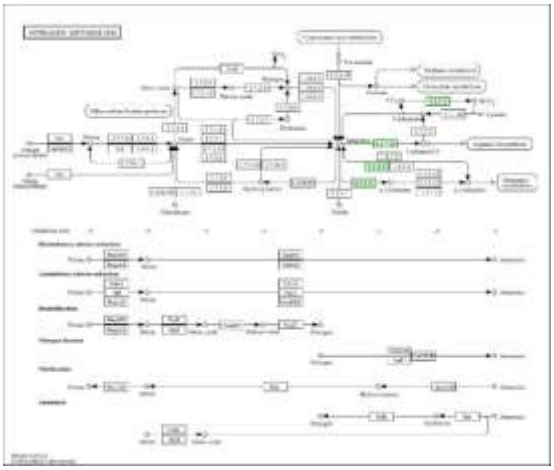


Fig 13: The image of Nitrogen metabolism pathway

1-DODECANOL

The KEGG pathway enrichment analysis for 1-Dodecanol identified several key pathways involving the target proteins.

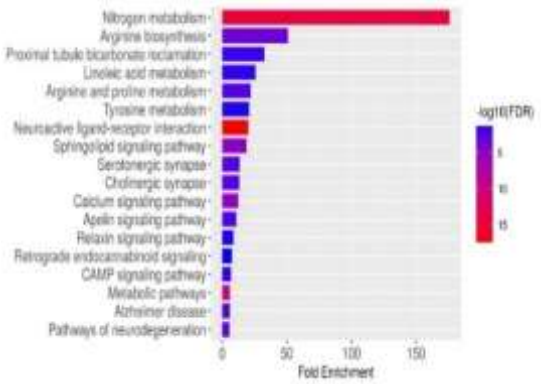


Fig 14: KEGG Pathway for 1-Dodecanol predicted targets

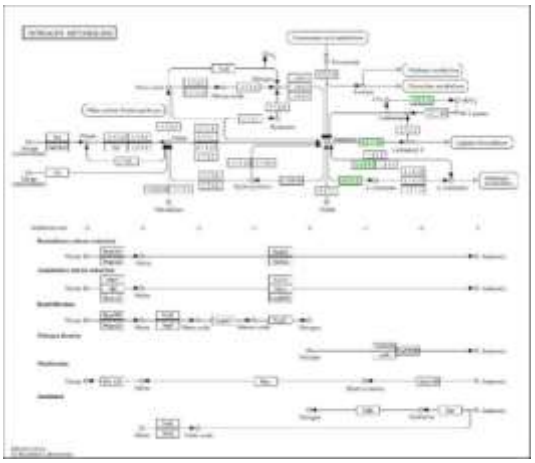


Fig 15: The image of Nitrogen metabolism pathway

Disease association analysis of compounds to show their effect on various diseases

In this study, Disease Alliance analysis was employed to explore the associations between the predicted protein targets and various diseases. This analysis integrates extensive bio clinical data, including genetic associations, clinical phenotypes, and epidemiological data, to identify potential links between the proteins of interest and specific diseases.

DICHLOROACETIC ACID

The Disease Alliance analysis for Dichloroacetic acid predicts associations with urethral obstruction, endometrial hyperplasia, bladder neck obstruction, hepatic encephalopathy, and renovascular hypertension.

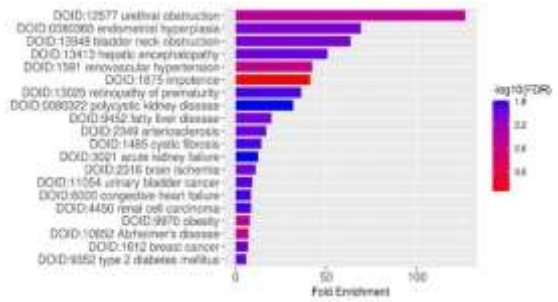


Fig 16: Disease Association for the predicted targets of Dichloroacetic acid

1-DODECANOL

The Disease Alliance analysis for 1-Dodecanol predicts associations with urethral obstruction, respiratory allergies, Alzheimer's disease, hepatic encephalopathy, and endometrial hyperplasia.

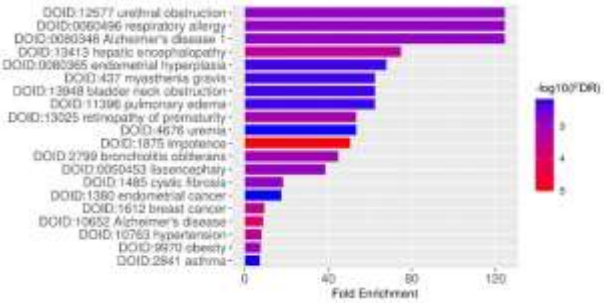


Fig 17: Disease Association for the predicted targets of 1-Dodecanol

DISCUSSION

This study successfully isolated *Aspergillus niger* from onion samples and identified its antimicrobial potential. The fungus was identified through macroscopic and microscopic characteristics, including white colonies turning dark brown and the presence of septate hyphae and conidia (Wei et al., 2022). The antimicrobial activity of the methanolic extract was evaluated against *Bacillus subtilis* and *Escherichia coli* using the well diffusion method. While the extract showed some antibacterial activity, the inhibition zones (2.3 mm for *B. subtilis* and 3.4 mm for *E. coli* at 100 µg/mL) were much smaller compared to the standard antibiotic Ciprofloxacin (14 mm and 13 mm, respectively). This suggests that although *Aspergillus niger* possesses antimicrobial properties, its activity is significantly weaker than conventional antibiotics like Ciprofloxacin, which inhibits bacterial DNA gyrase and topoisomerase IV (Natarajan et al., 2010; Al-Shaibani et al., 2013). This lower potency implies that while fungal extracts may offer a natural alternative, they may not be suitable as standalone treatments and would require optimization or combination with other agents to match the efficacy of established antibiotics.

GC-MS analysis identified a range of bioactive compounds, including oxalic acid esters, halogenated compounds, and alcohols (Hameed et al., 2015; Siddiquee et al., 2012; Niazi et al., 2023). These findings are consistent with previous reports on the diverse secondary metabolites produced by *Aspergillus niger* (Yu et al., 2021; Nielsen et al., 2009; Roy et al., 2021). A novel aspect of this study is the integration of GC-MS profiling with ADME (Absorption, Distribution, Metabolism, and Excretion) and protein–protein interaction (PPI) analyses, providing a multi-dimensional understanding of the pharmacological potential of *A. niger*-derived compounds. This combined approach is relatively underreported in previous literature and contributes new insights into the therapeutic potential and mechanism-based targeting of fungal metabolites.

ADME analysis revealed that several compounds, including dichloroacetic acid and 1-dodecanol, exhibit moderate drug-likeness properties (Abdelgawad et al., 2022; Mohamed et al., 2022). These results suggest potential for further development as antimicrobial agents. Protein-protein interaction (PPI) analysis and Gene Ontology (GO) enrichment indicated that these compounds may influence critical biological functions, such as immune regulation, signal transduction, and metabolic processes (Chaudhary et al., 2019; Zheng et al., 2021). These findings align with broader studies emphasizing the therapeutic relevance of fungal secondary metabolites (Bills and Gloer, 2016; Ramírez-Rendon et al., 2022).

Despite these promising results, these results set the baseline for future research. The antimicrobial testing was limited to only two bacterial strains; therefore, expanding the scope to include a broader range of pathogens is recommended (Wei et al., 2022). Furthermore, while GC-MS identified multiple compounds, isolating and testing these compounds individually will provide a clearer understanding of their bioactive roles (Hameed et al., 2015; Elfita et al., 2012). Toxicity profiling and in vivo efficacy studies will also be essential to ensure their therapeutic safety and relevance (Ortega et al., 2021; Hashem et al., 2023).

Future research should focus on expanding antimicrobial testing, isolating and characterizing individual bioactive compounds, and conducting in vivo studies to confirm therapeutic potential. Investigating molecular mechanisms and synergistic interactions with standard antibiotics could pave the way for the development of new drugs derived from *Aspergillus niger* (Singab et al., 2023; Toghueo et al., 2016; Bai et al., 2023).

CONCLUSION

In conclusion, this study thoroughly examined *Aspergillus niger*, focusing on its isolation, characterization, and bioactive compounds. The initial phase involved cultivating the fungus under controlled conditions, optimizing culture methods for maximum metabolite yield. The identification and morphological characterization, using Lacto Phenol Cotton Blue (LPCB) staining, confirmed the fungus as *Aspergillus niger*, revealing key features such as septate hyphae, globular vesicles, and biseriate phialides. The antimicrobial activity of *A. niger* extracts was tested against *Bacillus subtilis* and *Escherichia coli*. The results showed mild antibacterial effects, particularly against *B. subtilis*, with optimal activity at a lower concentration (50 µg/ml). Gas Chromatography-Mass Spectrometry (GC-MS) identified sixteen bioactive compounds, with potential biological interactions predicted through TargetNet. Experimental validation of these interactions is necessary for practical applications. Additionally, protein-protein interaction analysis suggested that Dichloroacetic acid (DCA) may be involved in oxidative stress and cell wall biosynthesis, while 1-Dodecanol may affect membrane dynamics and lipid metabolism. Gene Ontology (GO) analysis indicated DCA's role in energy metabolism and nucleotide synthesis, while 1-Dodecanol impacts lipid signaling and cellular communication. Both metabolites were linked to nitrogen metabolism, with potential therapeutic implications for metabolic disorders. This research Enhances the understanding of *A. niger*'s antimicrobial potential, metabolic functions and health-related implications, providing a foundation for future studies in biotechnology and medicine.

Declaration by Authors

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