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Antimicrobial potential of *Aspergillus fumigatiaffinis* and *A. sclerotiorum*: Insights from *in vitro* and molecular docking investigations

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Abstract

This study was conducted to evaluate the antimicrobial activity of metabolites produced by two *Aspergillus* species: *A. fumigatiaffinis* and *A. sclerotiorum*, against six bacterial strains and a yeast. An extraction of metabolites was carried out using three solvents, after selection of the best solvent, the obtained organic extracts were exposed to extreme conditions to test their stability. furthermore, three culture media with different compositions were used to select the best medium. The obtained results showed that the two *Aspergillus* species have interesting antimicrobial activity. Chloroform proved to be the best solvent for the extraction of bioactive metabolites. Additionally, the stability study showed that the majority of active extracts retain their activity after heat treatment (up to 100 °C) and exposure to light. However, the most suitable medium for antimicrobial activity was PDB. Molecular docking techniques were employed to explore the interactions between secondary metabolites from *Aspergillus* strains and the gyrase enzyme of *Staphylococcus aureus*, which was further supported by *in vitro* tests demonstrating strong antimicrobial activity of *Aspergillus* strains extracts against this bacterium. Docking analysis revealed compelling binding affinities of selected *Aspergillus*-derived secondary metabolites to the gyrase enzyme active site, characterized by diverse interaction patterns. These interactions offer insights into potential inhibitory effects on the gyrase enzyme, and suggest promising avenues for the development of therapeutic interventions against *S. aureus* infections.

Keywords: *Aspergillus fumigatiaffinis*; *Aspergillus sclerotiorum*; antimicrobial activity; molecular docking; secondary metabolites

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Introduction

From the beginning until now, humanity has always faced the problem of spreading pathogens responsible for a wide range of animal and human diseases, as well as the difficulty of their treatment. Through a succession of observation and the work of many researchers including Louis Pasteur, Joseph Joubert, Ernest Duchesne, the quest led to the discovery of antibacterial and antifungal substances, since this discovery, antimicrobial substances represent a potential source in the pharmaceutical field to treat various diseases (Moroh, 2013). The ability of microbes to develop resistance to a wide range of antimicrobial molecules evolves gradually over time, nowadays it concerns all pathogenic microbes such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, etc. Hence the need to develop new alternatives to fight these infections and to control the spread of resistant pathogens (Signoretto *et al*., 2012). Fungi have a huge potential to produce secondary metabolites with a very high antibacterial and antifungal power. These bioactive molecules are important actors in the microbial world (Pasqualotto, 2009). Furthermore, *Aspergillus* species are renowned for their ability to produce a wide array of secondary metabolites, many of which exhibit potent antimicrobial properties. These bioactive compounds encompass diverse chemical structures and mechanisms of action, making them valuable candidates for pharmaceutical development (Pasqualotto, 2009). Specifically, compounds such as polyketides, terpenoids, and alkaloids derived from *Aspergillus* strains have demonstrated remarkable efficacy against a spectrum of bacterial and fungal pathogens (Sadorn *et al*., 2016; Orfali *et al*., 2021; Balasubramaniyam *et al*., 2023; Zhu *et al*., 2023).

Moreover, targeting the gyrase enzyme of *Staphylococcus aureus* holds significant therapeutic promise due to its pivotal role in DNA replication and transcription. Inhibition of gyrase activity disrupts essential cellular processes, thereby impairing bacterial viability and proliferation. Given the alarming rise in multidrug-resistant *S. aureus* strains, the development of novel agents that specifically target gyrase represents a crucial strategy for combating infections caused by this notorious pathogen (Saiki *et al*., 1999).

The primary aim of this study was to evaluate the antimicrobial potential of secondary metabolites produced by two *Aspergillus* strains, *Aspergillus fumigatiaffinis* and *Aspergillus sclerotiorum*. Additionally, molecular docking techniques were employed to investigate the interactions between these secondary metabolites and the gyrase enzyme of *Staphylococcus aureus*, providing insights into their potential as therapeutic agents against *S. aureus* infections.

Materials and Methods

Microorganisms

The two fungal strains used in this work are *Aspergillus fumigatiaffinis* (MH109540) which was isolated from soil taken from an oasis palm grove in the northern region of Laghouat (situated 400 km south of Algiers, Algeria) and *Aspergillus sclerotiorum* (MH109547) which was isolated from a thermal spring floor in Teleghma region (located 298 km north-eastern of Algiers). This study is focused on exploring the antimicrobial properties of these two fungal strains against six bacterial strains: three Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Enterococcus faecalis*), and three Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae*), and one fungal strain (*Candida albicans*).

Antimicrobial activity determination

Preparation of suspensions

This test is performed to determine the antimicrobial activity of the two fungal strains. In physiological water, bacterial and fungal suspensions are prepared from viable young colonies that had been previously reactivated and then incubated at 37 °C for 24 to 48 hours. The turbidity of the suspensions was adjusted by visual comparison with a 0.5 Mc Farland solution (An optical density equal to 0.2 at 650 nm) (Sadrati *et al*., 2023).

Agar cylinder technique

Agar cylinders approximately 6 mm in diameter were cut using a sterile cookie cutter from 14-day-old fungal cultures. These were obtained by transplanting the strains on Potato Dextrose Agar (PDA: 200 g of potato, 20 g dextrose, 20 g agar and 1000 mL distilled water) medium and incubating at 28 °C. The agar cylinders were then placed on Petri dishes containing the Müller Hinton medium (2 g of beef extract, 17.5 g acid hydrolysate of casein, 1.5 g starch, 17 g agar and 1000 mL distilled water), previously seeded with the bacterial strains, while for the antifungal test, the agar cylinders were placed on Petri dishes containing Sabouraud agar (40 g of dextrose, 10 g peptone, 15 g agar and 1000 mL distilled water), previously seeded with the *C. albicans* strain. The dishes were placed at 4 °C for 4 hours and then incubated at 37 °C for 24 hours (Boughachiche, 2012). The antimicrobial activity was then determined by measuring the diameters of the inhibition zones that appear around the cylinders.

Production and extraction of bioactive molecules by different solvents.

Eight discs of each seven-days-old culture were placed in 500 mL vials containing 100 mL of Potato Dextrose Broth (PDB: 200 g of potato and 20 g dextrose and 1000 mL distilled water) medium. They were then incubated at 28 °C for 14 days. After this, the secondary metabolites were then extracted by adding a volume of solvent equivalent to that of the medium. Three solvents of different polarities were used: methanol, chloroform and cyclohexane. The mixture was homogenized, then the biomass was filtered using a Whatman N°1 paper. The resulting mixtures were then placed in a decanting bulb and the organic phase was recovered. In addition, the aqueous phase was treated several times, with the solvent to be evaporated at 40 °C using a rota-vapor (Zerroug, 2011; Ghorri, 2015). The extracts were prepared at a concentration of 100 mg/mL, by dissolution in dimethylsulfoxide (DMSO).

Well method

On Müller Hinton agar seeded with test bacteria aged between 18 to 24 hours and on Sabouraud agar seeded with *C. albicans*, 3 mm diameter wells were perforated with a cookie cutter and 20 µL of each extract was added to each well. The dishes were left at room temperature for 30 minutes, and then incubated at 37 °C for 24 h (Prabavathy and Valli, 2012; Bramki *et al.*, 2019). Antimicrobial activity was determined by measuring the inhibition zone around each well (a clear zone around the well). For each extract, three replicate trials were performed against each organism.

Stability test of the extracts

This test is used to determine the stability of bioactive compounds under different conditions. For this purpose, aliquots of 400 µL of active organic extracts were prepared at a concentration of 100 mg/mL and separately exposed to different temperatures (50 °C, 70 °C, 100 °C) for 30 min as well as to periods of darkness and light (3350 lumen) for 15 days (Boughachiche, 2012). A control of 400 µL of each extract is prepared in the same previous way but without treatment. The obtained results from the treated samples were compared with those of the controls. The extracts were then tested by the well method.

Production and extraction of secondary metabolites from different fermentation media

In order to select the most suitable medium for the production of secondary metabolites with antimicrobial activity, the fermentation is carried out on three different liquid media Czapek Dox (20 g of sucrose, 2 g sodium nitrate, 1 g dipotassium phosphate, 0.5 g magnesium sulfate, 0.5 g potassium chloride, 0.01 g ferrous sulfate and 1000 mL distilled water), Yeast Peptone Dextrose Broth (YPD: 10 g of yeast extract, 20 g peptone, 20 g dextrose and 1000 mL distilled water) and PDB in the same way as before (Zerroug, 2011; Ghorri, 2015). The extraction of secondary metabolites is done by the solvent that gave the best result. The antimicrobial activity test was performed using the same previous method (wells).

Statistical analysis

All measurements were taken three times for each treatment. Statistical analyses of the data were performed using the SPSS (version 25.0) software. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test for multiple comparisons. Differences were considered significant at $P < 0.05$.

Investigating secondary metabolite interactions with S. aureus DNA gyrase using molecular docking

In this study, we employed molecular docking techniques to elucidate the binding interactions between secondary metabolites from various *Aspergillus* strains and the DNA gyrase enzyme (PDB code: 3U2D) of *S. aureus*. The choice of *S. aureus* for docking analysis was guided by *in vitro* tests demonstrating the superior antimicrobial activity of *Aspergillus* strains extracts against *S. aureus*. The gyrase enzyme was selected as a therapeutic target due to its vital role in DNA replication, making it an attractive target for novel drug development.

Ligands preparation

The 3D structures of secondary metabolites from *Aspergillus* strains were obtained from the chemical database Pubchem and preprocessed to ensure proper geometries and energy minimization. Ligands were prepared using appropriate software tools, including ligand energy minimization and optimization, as required by AutoDock Vina.

Receptor preparation

The crystal structure of the DNA gyrase enzyme (3U2D) was retrieved from PDB database. Prior to docking analysis, the protein structure underwent energy minimization, removal of water molecules and non-essential co-factors, and assignment of atomic charges.

Molecular docking

Molecular docking was performed using AutoDock Vina (Trott *et al*., 2010), a widely employed molecular docking software. Ligands were docked into the active site of the gyrase enzyme. The grid box was defined around the active site, ensuring comprehensive exploration of ligand binding modes. Docking parameters were optimized for maximum accuracy and efficiency, including exhaustiveness and search space dimensions.

Visualization and analysis

Docking results were visualized and analyzed using Discovery Studio, providing insights into ligand-binding conformations, interactions, and binding energies. 3D visualization highlighted ligand binding orientations within the active site, while 2D interaction diagrams illustrated specific interactions such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions.

Results and Discussion

Antimicrobial activity determination

Antibacterial activity results revealed that *A. fumigatiaffinis* and *A. sclerotiorum* acted as excellent antibacterial agents against both Gram-positive and Gram-negative bacteria, and against the fungal strain *C. albicans* (Figure 1).

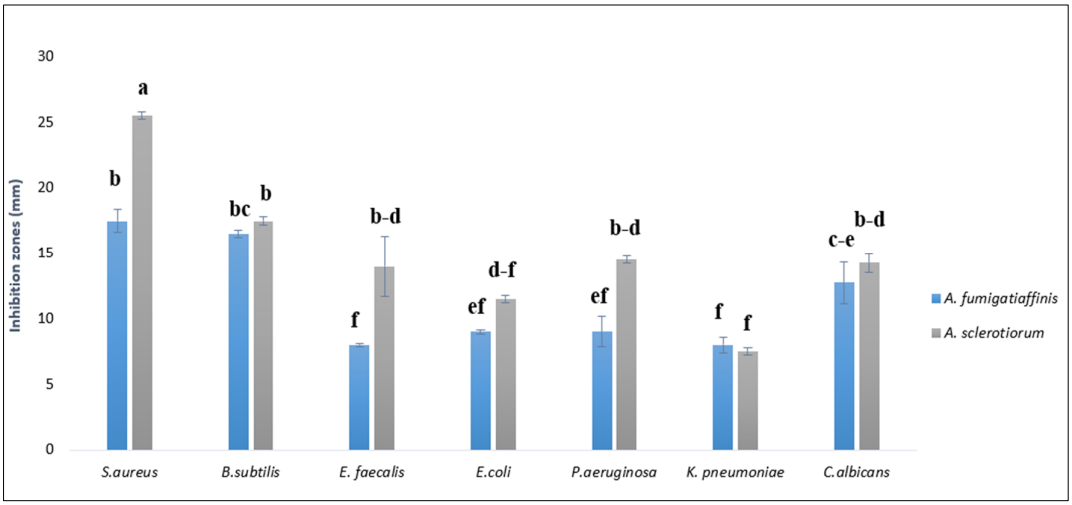

Figure 1. Antimicrobial activity in terms of inhibition zone (mm) resulted by testing the two fungal species (*A. fumigatiaffinis* and *A. sclerotiorum*) against *S. aureus*, *B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans* using the agar cylinders technique

Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

Indeed, the results showed that *A. fumigatiaffinis* and *A. sclerotiorum* strains exhibited maximum bacterial growth inhibition against *S. aureus*. Inhibition zones were found to be the following; 25.0 and 17.0 mm for *A. sclerotiorum* and *A. fumigatiaffinis* respectively. Similar patterns were observed in the case of *S. aureus*, *B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and against the fungal strain *C. albicans*, where the maximum inhibition zone was exhibited by *A. sclerotiorum* followed by *A. fumigatiaffinis*. In contrast, both fungal strains *A. fumigatiaffinis* and *A. sclerotiorum* showed similar inhibition zones against *K. pneumoniae*.

In agreement with our results, Al-Shaibani *et al*. (2013) reported that *A. niger* had a significant inhibitory effect against; *P. aeruginosa*, *S. aureus*, *S. epidermidis* and *Bacillus* sp. Ruma *et al*. (2013) found that *A. fumigatus* extract has an important antibacterial activity against *Shigella flexneri*, *B. subtilis*, *E. coli*, *K. pneumoniae* and *S. aureus*. Kalyanasundaram *et al*. (2015) found that *A. terreus* strain has a good antibacterial activity against *S. typhi*, *S. aureus*, *V. cholera*, *E. coli*, *K. pneumoniae*, *S. paratyphi* and *K. oxytoca*. Padhi *et al*. (2017), showed that *A. tubingensis* has a remarkable antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *S. flexneri* and *K. pneumoniae*. As well as Ogbole *et al*. (2017), revealed that *A. tamarii* has a significant antibacterial activity against a wide range of bacterial strains including *S. typhi*, *S. aureus*, *B. subtilis* and *E. coli*.

As for test germs, the antibacterial activity against Gram-positive bacteria appears to be more important than that against Gram-negative bacteria. This is related to the results obtained by Prabavathy and Valli (2012). Indeed, these results can be explained by the presence of an external membrane in gram-negative bacteria that make them resistant (Breijyeh *et al*., 2020).

Choice of the best solvent for the extraction of molecules

The choice of the best extraction solvent depends on the antimicrobial activity of the fungal extracts obtained. The results of the well technique (Figure 2) showed that both fungal strains had a good antimicrobial activity against five out of seven strains tested; *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae* and *C. albicans*.

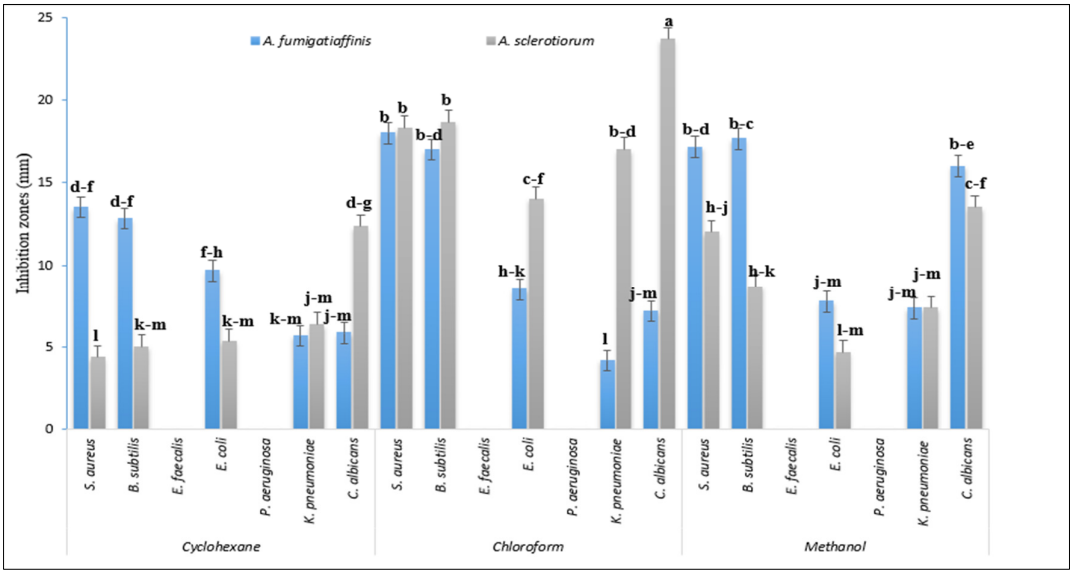

Figure 2. The effect of different solvent extracts on the antimicrobial activity of the two fungal strains (*A. sclerotiorum* and *A. fumigatiaffinis*)

Vertical bars represent standard error (n = 3). Bars sharing similar letter(s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

For the *A. fumigatiaffinis* strain, the highest antibacterial potential was observed by the chloroformic and methanolic extracts against the two bacteria *S. aureus* and *B. subtilis*, with average inhibition zones diameters ranging from 17.0 to 18.0 mm. Followed by the effect of the cyclohexane extract on the same bacteria, *S. aureus* and *B. subtilis* with diameters of 13.5 and 12.8 mm respectively. However, the strains *E. coli* and *K. pneumoniae* showed a less important sensitivity towards the three organic extracts with inhibition zones ranging between 4.2 to 9.7 mm. Moreover, the three extracts were inactive against the *E. faecalis* and *P. aeruginosa* tested strains.

Regarding antifungal activity against *C. albicans*, the most significant effect was obtained using the methanol extract, with an inhibition zone of 16.0 mm in diameter, followed by the chloroform and cyclohexane extracts with inhibition zones of 7.2 and 5.8 mm in diameter respectively.

Concerning the antibacterial activity of the *A. sclerotiorum* species, the most significant inhibition zones were marked by the chloroform extract, especially against *B. subtilis* and *S. aureus* bacteria with diameters of 18.7 and 18.3 mm respectively. *K. pneumoniae* and *E. coli* bacteria showed slightly lower sensitivity, with inhibition zones ranging from 17.0 and 14.0 mm in diameter. However, both extracts; methanolic and cyclohexane demonstrated a relatively weak antibacterial effect against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae* bacteria with diameters ranging from 4.3 to 12.0 mm. For the activity against *C. albicans*, the three tested extracts gave a positive result. The greatest antifungal potential was shown by the chloroformic extract with an inhibition zone of 23.7 mm in diameter, followed by the methanolic extract with a diameter of 13.5 mm and the cyclohexane extract with a diameter of 12.3 mm.

Based on the obtained results, the most effective solvent for the extraction of secondary metabolites with antimicrobial activity is chloroform. In second place are methanol extracts, followed by cyclohexane extracts

with low activity against most microbial strains. This result is consistent with those of Kitouni (2007) and Bramki *et al*. (2019), who found that chloroform, is among the best solvents used to extract bioactive molecules.

Organic solvents play an important role in the extraction of bioactive secondary metabolites as raw compounds starting from culture media according to their polarity, so secondary metabolites are extracted differentially (Kamat *et al*., 2020; Sadrati *et al*., 2023).

Methanol is a highly polar solvent. Chloroform is semi-polar. While cyclohexane is non-polar. From this, we can conclude that the obtained extracts contain several secondary metabolites according to their polarity. There are various reports according to which the antimicrobial activity depends on the used solvent, the structure of compounds found in in the extracts and the studied strain. The raw extracts recovered by different organic solvents have different chemical compounds in different quantities and therefore they act differentially on microbial strains (Schügerl, 2013; Chaudhari *et al*., 2014; Padalia and Chanda, 2015; Stancu, 2016).

Stability test

To test the stability of the extracts, these last ones were exposed to unfavorable conditions namely; high temperatures, intense light as well as darkness. The results of well method showed that, antimicrobial molecules of almost all active extracts kept their activity after treatment (Figures 3, 4, 5, 6, 7 and 8).

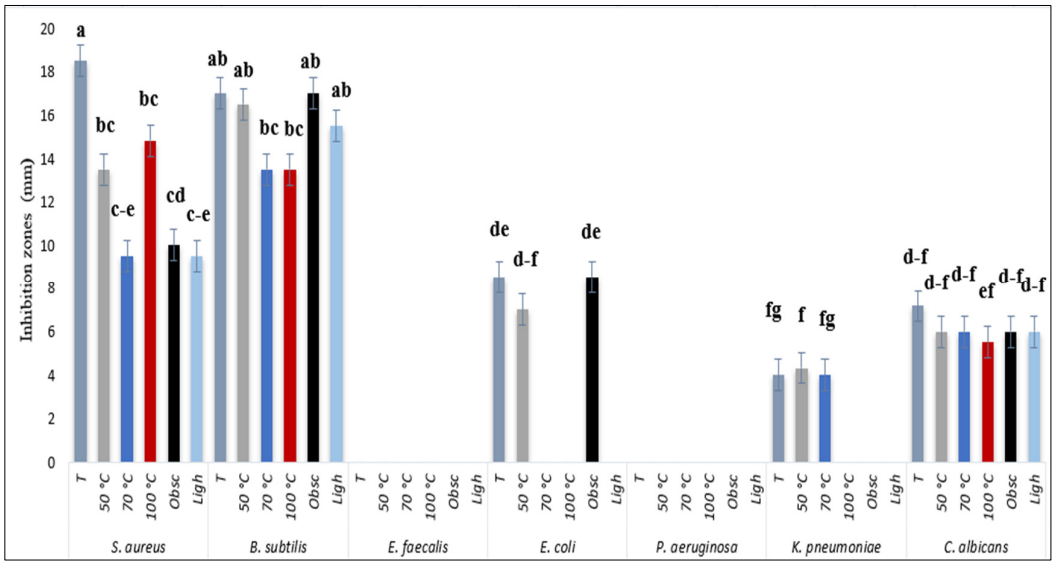

Figure 3. Antimicrobial effect of chloroformic extract of *A. fumigatiaffinis* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

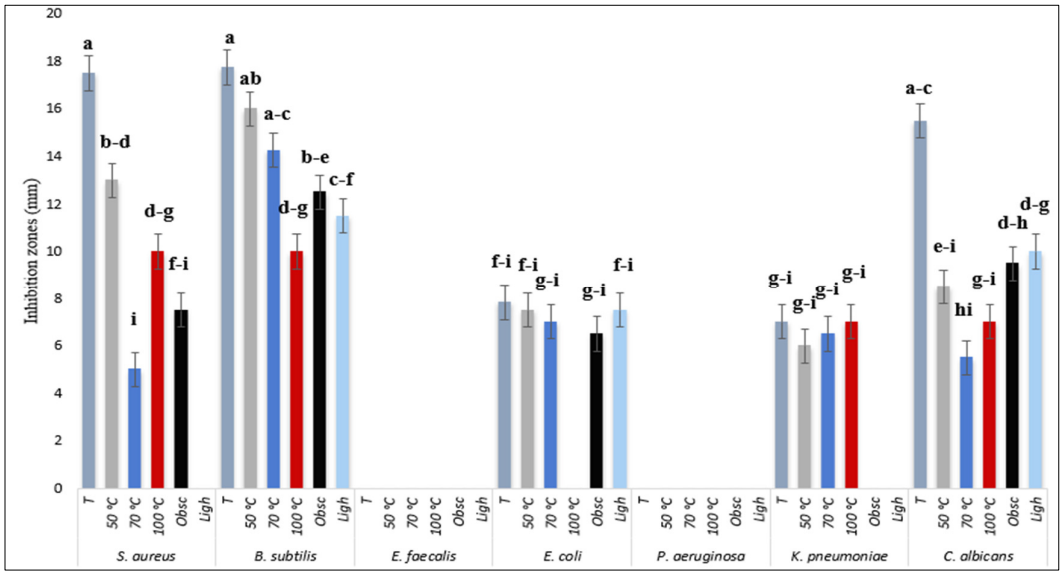

Figure 4. Antimicrobial effect of methanolic extract of *A. fumigatiaffinis* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

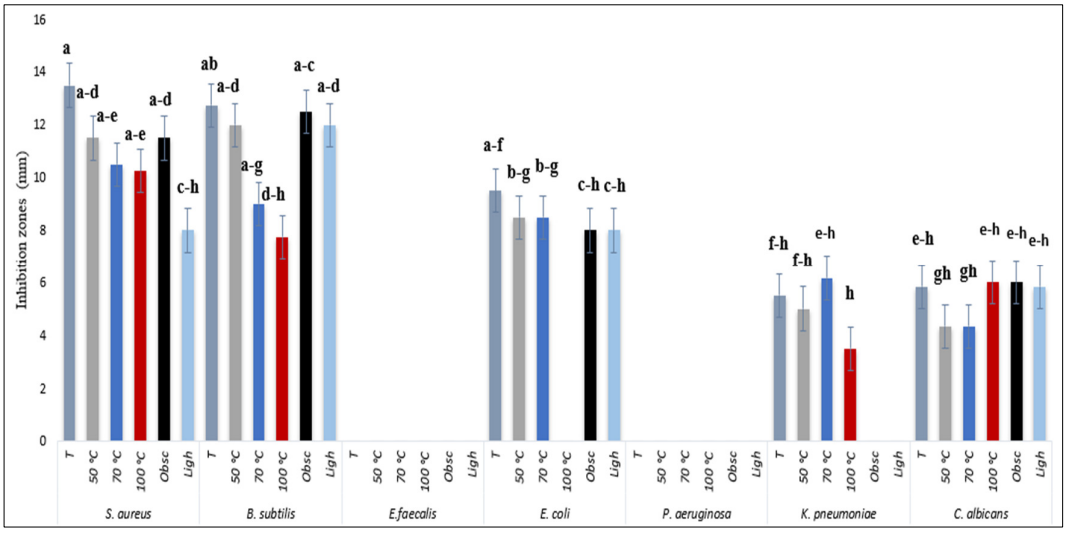

Figure 5. Antimicrobial effect of cyclohexane extract of *A. fumigatiaffinis* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

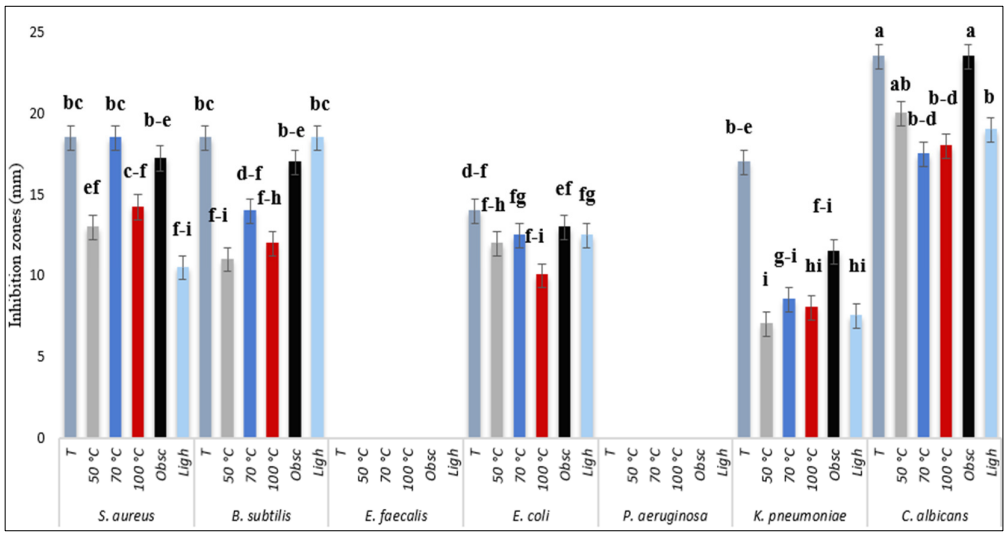

Figure 6. Antimicrobial effect of chloroformic extract of *A. sclerotiorum* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

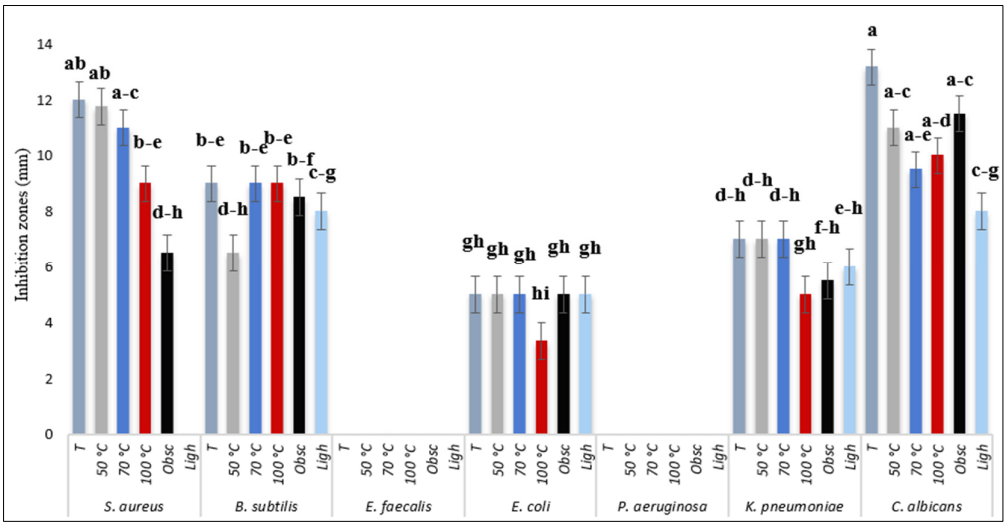

Figure 7. Antimicrobial effect of methanolic extract of *A. sclerotiorum* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

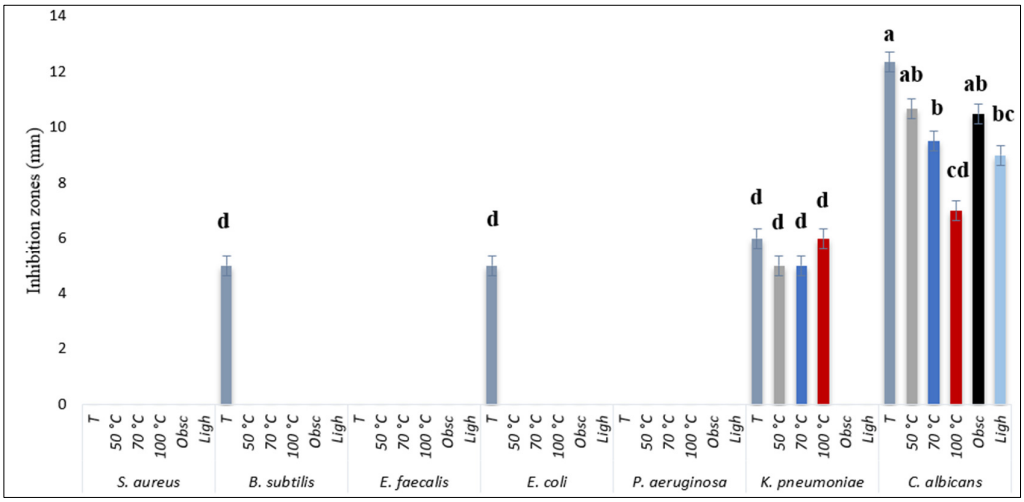

Figure 8. Antimicrobial effect of cyclohexane extract of *A. sclerotiorum* after heat and light treatment
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

For the fungal strain *A. fumigatiaffinis*, cyclohexane extract lost its activity only against *E. coli* species after treatment at 100 °C, and against *K. pneumoniae* species after exposure to light and darkness. In addition, the chloroformic extract, lost its activity only against *E. coli* after exposure to 70 °C, 100 °C and light, and against *K. pneumoniae* after exposure to 100 °C, darkness, and light. The methanolic extract of the same fungal species retained its antimicrobial activity, except for the *S. aureus* strain after exposure to light, *E. coli* strain after heat treatment at 100 °C, and *K. pneumoniae* strain, after exposure to light and darkness (Figures 3, 4 and 5).

Regarding the fungal strain *A. sclerotiorum*, organic extracts show more or less stable activity after treatment. For the cyclohexane extract, the antimicrobial potency was remarkably affected especially against *B. subtilis*, *E. coli*, and *K. pneumoniae* strains only after exposure to light and darkness. In fact, the chloroformic extract showed resistance to the different treatments where the majority of the inhibition diameters values recorded being very close to each other for all the test bacteria and almost equivalent to those of the untreated extracts. Moreover, the methanolic extract has retained its antimicrobial potential where the diameters of the inhibition zones are close to those obtained by the control extract, except for the bacterium *S. aureus* after exposure to light (Figures 6, 7 and 8).

The study of bioactive molecules stability shows that the majority of active extracts retain their activity after heat treatment (up to 100 °C) and exposure to light for two weeks. Which translates to the presence of stable molecules in the raw extracts of the studied strains. These results are consistent with those reported by Singh *et al.* (2010), who worked on the thermostability of an *A. terricola* metabolite and found that it was not affected during incubation at 70 °C for 2.5 h. Similarly, Gasparetti *et al*. (2010), who worked on a metabolite of *A. oryzae*, and showed that it has good thermostability up to 60 °C.

Choice of the optimum medium for the production of bioactive substances

After selecting the most suitable solvent for the extraction of secondary metabolites, a second fermentation was carried out on three liquid media (Czapek Dox, YPD and PDB) to select the most efficient culture medium promoting the production of antimicrobial substances. The well technique results show that all extracts have the ability to inhibit the growth of at least one test strain. The diameters of the inhibition zones

varied from one culture medium to another and from one test bacterium to another. However, both strains, *E. faecalis* and *P. aeruginosa* showed resistance to all organic extracts (Figure 9).

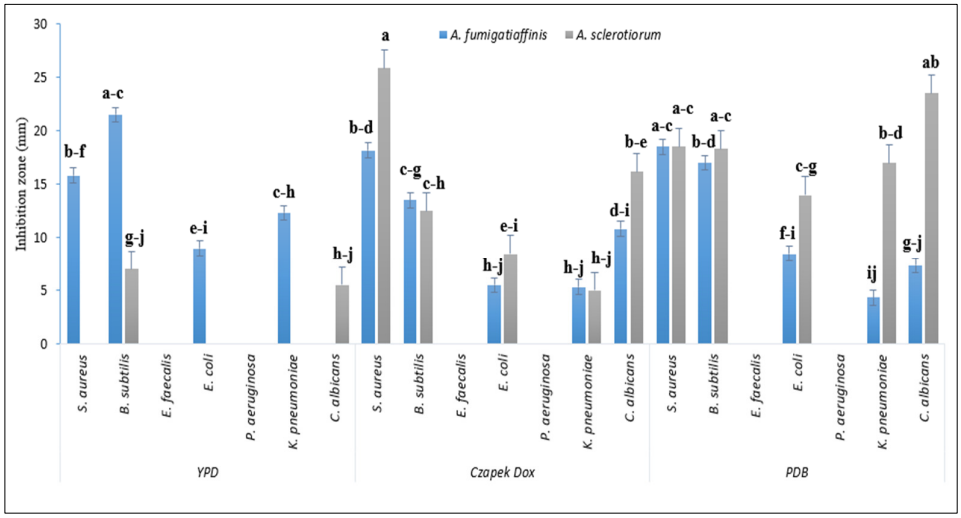

Figure 9. Effect of different culture media on antimicrobial activity of fungal strains
Vertical bars represent standard error (n = 3). Bars sharing similar letter (s) are statistically non-significant at $p \leq 0.05$ according to Tukey's HSD test.

For the *A. fumigatiaffinis*, the highest antibacterial activity was observed by the YPD medium extract against *B. subtilis* with a diameter of 21.5 mm, followed by the effect of extracts from PDB and Czapek-dox media against the *S. aureus* bacterium with diameters of 18.5 mm and 18.2 mm respectively. Then effects of the extracts of the PDB medium on *B. subtilis* with a diameter of 17.0 mm, YPD medium on *S. aureus* with a diameter of 15.8 mm, and Czapek-dox medium on *B. subtilis* with a diameter of 13.5 mm. In addition, the antibacterial potential of all extracts is less important against *E. coli*, and *K. pneumoniae* bacteria, where the inhibition zones ranged from 4.3 mm to 12.3 mm. On the other hand, only extracts coming from Czapek-dox and PDB media showed activity against the yeast *C. albicans* with diameters of 10.8 mm and 7.3 mm respectively.

For the *A. sclerotiorum*, the extract from Czapek-dox medium showed a very high antibacterial potential against *S. aureus* with an inhibition diameter of 25.8 mm, followed by an antifungal effect against *C. albicans* with a diameter of 16.2 mm, then a less important effect against *B. subtilis*, *E. coli*, and *K. pneumoniae* bacteria with diameters ranging from 5.3 mm to 12.5 mm. In addition, the extract from PDB medium marked an important antibacterial activity against the tested strains ranging from 14.0 mm to 18.7 mm, as well as a very high antifungal activity against *C. albicans* with an inhibition zone of 16.5 mm. However, the YPD medium extract showed a very low antimicrobial activity with an inhibition zone diameter of 7mm against *B. subtilis*, and 5.5 mm against *C. albicans* only.

Based on the previous results, it can be concluded that the most efficient medium for the production of secondary metabolites with antimicrobial activity is the PDB medium, with the exception of the *A. sclerotiorum* extract, coming from Czapek-Dox medium, which showed an excellent effect against the *S. aureus* bacterium. Our results can be explained by the fact that fungi have a very high preference for one or more chemical compounds of their appropriate medium. Furthermore, the performance of the PDB medium is explained by the presence of starch, this slowly metabolizable carbon source is often good for antibiotic production (Awad, 2005). Based on the obtained results and other literature data, it is clear that the production of bioactive secondary metabolites of different fungal species and their antimicrobial potency varies considerably depending

on the species and their nutritional status. According to the results of Jawad and Zafar (2017), the chemical composition of the culture media (in sources of carbon, nitrogen, phosphorus, sulphur, iron, etc.) is one of the most important factors that influence the production of secondary metabolites. In addition, Gesheva *et al*. (2005) mentioned that carbon and nitrogen are the most important components of culture media; the selection of their sources and concentrations plays a crucial role in the growth of fungi and in their production of primary and secondary metabolites. In addition, Shahid and Nadeem (2015), indicated that sucrose and yeast extract were the most favorable sources of carbon and nitrogen for mycelium growth.

Investigating Secondary Metabolite Interactions with S. aureus DNA gyrase using molecular docking

Molecular docking plays a pivotal role in understanding the interactions between ligands and target proteins, thereby aiding in the discovery of potential therapeutic agents. The current study involved an in-depth molecular docking investigation of the interactions between a selection of secondary metabolites from diverse *Aspergillus* strains and the DNA gyrase enzyme (3U2D) associated with *S. aureus*. The binding energies, along with specific interaction patterns, were meticulously analyzed to unveil potential inhibitory effects of these metabolites on the target enzyme.

The docking results presented in Table 1 demonstrate the interactions between selected secondary metabolites from *Aspergillus* strains and the gyrase enzyme of *S. aureus*. Notably, these interactions are crucial indicators of the potential inhibitory effects of these compounds on the target enzyme.

Table 1. Best docking results of *Aspergillus* selected secondary metabolites with *S. aureus* DNA gyrase (3U2D)

Compounds	Binding energy (Kcal/mol)	Hydrogen interactions (distance Å)	Hydrophobic interactions	Van der Waals Interactions	Electrostatic interactions
Co-crystallized inhibitor 4-bromo-5-methyl-N-[1-(3-nitropyridin-2-yl) piperidin-4-yl]-1H-pyrrole-2-carboxamide	-7.5	Glu58, Pro87	Ile175, Val79, Ile51, Asn54, Ile86, Pro87	Arg144, Gly85, Thr173, Leu103, Asp81, Ile102	Arg84
Chaetoglobosin B	-9.8	Asp57, Asn54, Asp81	Ile86	Pro87, Glu58, Ile102, Ser55, Thr173, Ile175, Ile51, Leu103	-
Butyrolactone I	-9.7	Gly85, Asp81, Thr173, Arg84, Pro87, Asn54	Ile51, Ile175, Ile102, Ile86	Leu103, Ser55, Gly172, Gly83, Asp57	Glu58
Versicolorin	-9.5	Asn54	Ile86 (3), Gly85	Ile102, Gly83, Glu58, Arg84, Gly172, Asp81, THR173, Ile175, Leu103, Ile51, Ser129	-
Asperazine	-9.2	Asn54 (2)	Ile51, Leu103, Pro87	Ile175, Ala98, Gln91, Ile102, Asp89, Arg144, Asp57, Ile86, Ser129, Gly85	Arg84, Glu58
Isoversicolorin C	-8.4	Asn54, Thr173	Ile175, Thr173, Ile86 (3)	Leu103, Ile102, Arg84, Glu58, Asp81, Ser55, Val79, Ile51	-

The docking results revealed significant binding affinities of selected secondary metabolites from *Aspergillus* strains with the gyrase enzyme of *S. aureus*. The observed interactions encompassed hydrogen bonding, hydrophobic contacts, and van der Waals forces, collectively contributing to the stability of the ligand-protein complexes. Compounds demonstrating potent binding interactions might exhibit inhibitory effects on the gyrase enzyme, suggesting potential therapeutic implications against *S. aureus* infections.

Notably, the binding energies demonstrated a range of values, with the most favorable interactions revealed by compounds such as chaetoglobosin B and butyrolactone I, displaying binding energies of -9.8 and -9.7 kcal/mol, respectively. These compounds exhibited intricate networks of hydrogen bonding with key residues including Asp57, Asn54, and Asp81, coupled with hydrophobic interactions involving Ile86 and various van der Waals contacts (Figures 10 and 11).

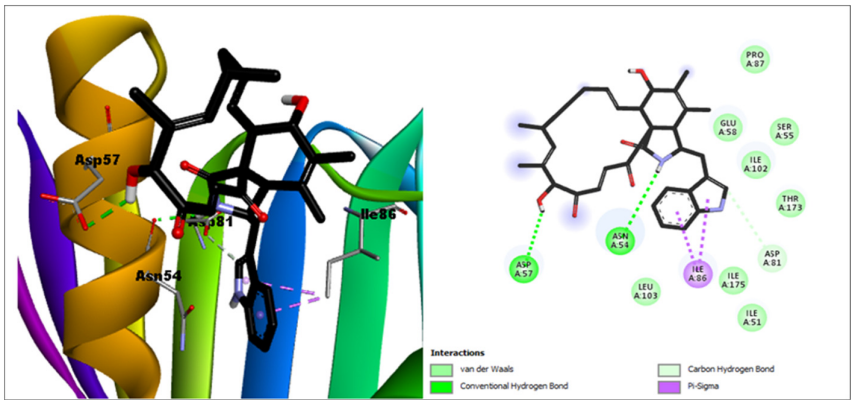

Figure 10. 3D and 2D interaction model of chaetoglobosin B binding to *S. aureus* gyrase active site

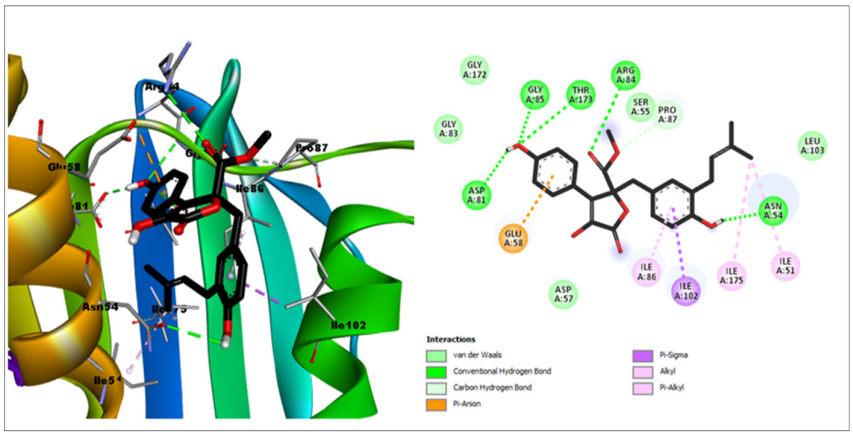

Figure 11. 3D and 2D interaction model of butyrolactone I binding to *S. aureus* gyrase active site

Similarly, the compound versicolorin exhibited a noteworthy binding energy of -9.5 kcal/mol, establishing pivotal hydrogen bonds with Asn54 and significant hydrophobic interactions (Figure 12). The interactions with residues Ile86 and Gly85 underscored its potential as a potent gyrase inhibitor. Moreover, compounds such as asperazine and isoversicolorin C demonstrated intriguing interactions as well, displaying binding energies of -9.2 and -8.4 kcal/mol, respectively. The former displayed multiple hydrogen bonds with Asn54 and distinctive hydrophobic interactions, emphasizing its potential as an inhibitory agent (Figure 13).

The latter, isoversicolorin C, showcased significant hydrophobic interactions with residues Ile175, Thr173, and Ile86, suggesting a potential inhibitory role against gyrase (Figure 14).

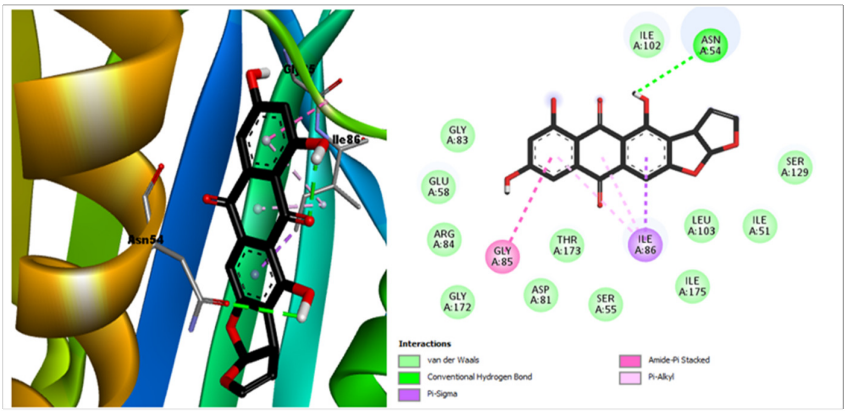

Figure 12. 3D and 2D interaction model of versicolorin binding to *S. aureus* gyrase active site

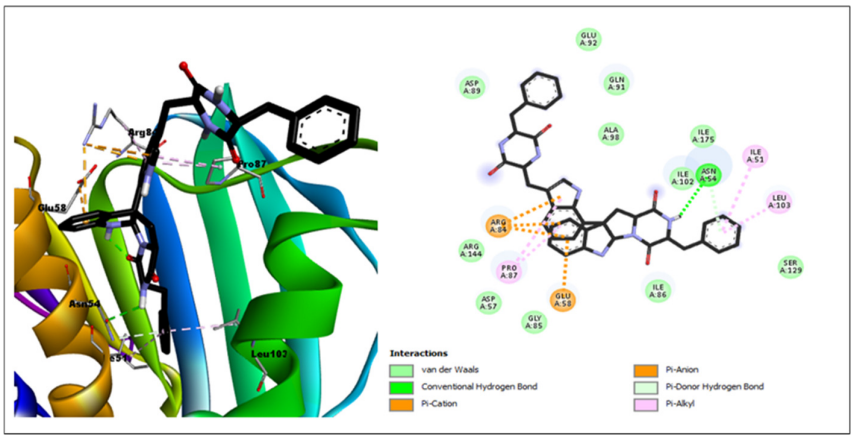

Figure 13. 3D and 2D interaction model of asperazine binding to *S. aureus* gyrase active site

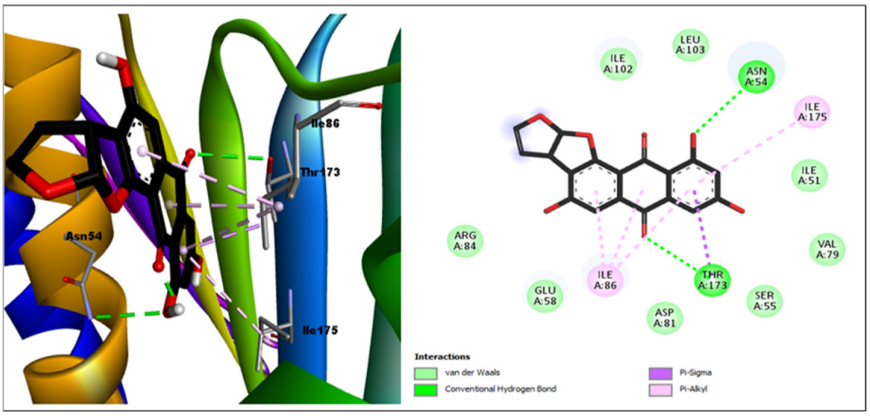

Figure 14. 3D and 2D interaction model of isoversicolorin C binding to *S. aureus* gyrase active site

These findings collectively underscore the diverse mechanisms through which secondary metabolites from *Aspergillus* strains interact with the gyrase enzyme of *S. aureus*. These interactions are indicative of their potential as antimicrobial agents, warranting further in-depth experimental validation to confirm their inhibitory effects and potential application as novel therapeutic agents against *S. aureus* infections.

Conclusions

The main objective of this work was to evaluate the antimicrobial activity of two fungal species; *A. fumigatiaffinis* and *A. sclerotiorum*. The results of the demonstration show that these last ones have a considerable antimicrobial effect against all tested strains. After the extraction of bioactive molecules, the results allowed to qualify chloroform as the most effective solvent. Moreover, the stability study reveals that high temperatures as well as darkness and light have no significant impact on the antimicrobial potential of the tested extracts. Furthermore, the results of fermentation on different media revealed that the nature of the source of carbon, nitrogen, and mineral source of culture media greatly influences the production capacity of bioactive metabolites in fungi. Molecular docking provided valuable insights into the interactions between *Aspergillus*-derived secondary metabolites and the gyrase enzyme of *S. aureus*. The results of this study shed light on potential drug candidates for further development and optimization in the quest to combat *S. aureus* infections. Our results elucidated the binding mechanisms between selected secondary metabolites and the gyrase enzyme, offering a rational foundation for the discovery of novel therapeutic agents.

Authors' Contributions

AB: Proposed the methodology, carrying out tests, recording results and manuscript writing; OB: Methodology, results analysis and manuscript writing; NR: Results analysis and interpretation; SG: Methodology; MB and BM: Carrying out tests. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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