

***In vitro* and *in silico* investigation of the antifungal activity of endophytic fungi against phytopathogenic fungi of tomato**

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Abstract

Plants are threatened by several diseases caused by phytopathogenic fungi. Melanin is an important pathogenicity factor in some fungal plant diseases. The enzyme 6,3,8-trihydroxynaphthalene reductase (3HNR) is implicated in the catalysis of the melanin biosynthesis in fungi. The chemical fungicide Phthalide acts by inhibiting this enzyme. But despite its efficacy, Phthalide can be detrimental to environmental health, hence the need to look for natural inhibitors to combat phytopathogenic fungi. This study aimed to screen the antifungal activity of some endophytic strains against phytopathogenic fungi of tomato. A total of 7 endophytic fungi were isolated and pre-identified from different parts of celery, parsley, mint, and coriander. On the other hand, five phytopathogenic fungal strains were isolated and pre-identified from tomatoes. The agar cylinder method showed that the endophytic fungi strains *Fusarium* and *Trichoderma* have significant inhibitory activity against four phytopathogenic fungi identified as *Alternaria* and *Penicillium*. Molecular docking was also used to study the inhibitory effect of some bioactive fungal compounds against the 3HNR enzyme. Drug-likeness and ADMET analyses were conducted on the selected chemicals to test their reliability and pharmaceutical efficacy. Phenylethyl alcohol interacts intensely with the binding site of the 3HNR receptor giving binding energy of -5.3 Kcal/mol, which is close to the co-crystallized ligand Phthalide. In addition, ADMET and pharmacokinetic analysis revealed that Phenylethyl alcohol verify the majority of the filters and pharmacokinetic properties necessary to select an effective antifungal molecule, including Lipinski's and Veber's rules.

Keywords: 3HNR; biological control; endophytic microorganisms; molecular docking; phytopathogenic fungi

Introduction

Plant pathogens such as fungi, bacteria, viruses, and nematodes cause huge damage to crops all over the world, reducing significantly the quality and quantity of agricultural products. Each year, these losses pose a major risk to global food production (O'Brien, 2017). More than 19,000 varieties of fungi are phytopathogenic and cause different types of diseases within crops (Lazarovits *et al.*, 2014). Plant diseases caused by phytopathogenic fungi include root decay, leaf spot, anthracnose, and rust, which are caused by the production of toxins, pigments, and enzymes resulting in a significant reduction in harvest quality (Iqbal *et al.*, 2018).

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Melanin is a high molecular weight pigment that represents a virulence factor for several pathogenic fungi (Jacobson, 2000). It is produced by oxidative polymerization and is widely distributed among microorganisms, plants, and animals (Bell and Wheeler, 1986). Several pathogenic fungi, including, *Aspergillus* sp., *Histoplasma capsulatum*, *Fonsecaea pedrosoi*, *Paracoccidioides brasiliensis*, *Alternaria* sp., *Sporothrix schenckii*, and *Fusarium* sp. have this molecule in their cell walls (Nosanchuk *et al.*, 2015).

The majority of fungal melanins are obtained from the precursor 1,8-dihydroxynaphthalene (DHN) and are referred to as DHN-melanins; the biosynthetic pathway that produces DHN is known as the pentaketide pathway (Bell and Wheeler, 1986). Malonyl-CoA acts as a precursor for the polyketide synthase (PKS1) enzyme, which catalyzes the first step in the biosynthesis pathway. PKS transforms malonyl-CoA to the first intermediate, 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN). Then, a particular reductase enzyme converts 1,3,6,8-THN to scytalone. Scytalone is dehydrated to 1,3,8-trihydroxynaphthalene, which is then reduced by a second reductase to vermeline, itself catalyzed by scytalone dehydratase, resulting in the intermediate 1,8-DHN, for which this pathway takes its name (Bulter *et al.*, 2005; Belozerskaya *et al.*, 2017).

The durability of a plant control method is defined as the persistence of its effectiveness in space and time. In developed countries, the use of chemical pesticides has increased considerably in recent decades (Usta, 2013). Biological control can be defined as the use of non-pathogenic antagonistic microorganisms to repress pathogenic organisms that cause disease in an environmentally friendly way (Khasa, 2017). Biocontrol of plant diseases entails using organisms and their products to suppress pathogens and help minimize their severity (Chaur, 1998). In recent years, biocontrol of pathogenic fungi has been considered as a potential control scheme because chemical control leads to the accumulation of environmentally hazardous residues, that can cause major environmental pollution (Nega, 2014).

Microbial agents have a variety of modes of action. These may require actual interactions between both the antagonist and the pathogen, interactions associated with roots or seeds, or interactions that occur freely in the soil. Furthermore, indirect interactions exist when the plant reacts to the presence of the antagonist, leading to mediated resistance or stimulation of plant growth (Pankhurst and Lynch, 2005).

Biological control outcomes whether from competition for nutrients and space, or by the capacity to produce metabolites that inhibit spore germination (fungistatic), kill cells (antibiosis), or adjust the rhizosphere, for instance by acidification the soil, so that pathogens cannot develop. Biocontrol can also result from direct interactions between both the pathogen itself and the antagonist, like in the case of mycoparasitism, which entails physical contact and the production of hydrolytic enzymes, harmful chemicals, and/or antibiotics that work in tandem with the enzymes (Bentez *et al.*, 2004).

Drug target screening by in silico modeling against plant pathogens can reduce the time and cost of searching for new compounds. In addition, in silico analysis allows the study of several features, such as protein-inhibitor interaction, inhibitor binding efficiency to the target protein, and the choice of the best confirmation of ligands, which fit the target protein by generating multiple orientations (Silva Jr *et al.*, 2017).

The main aim of this research was to assess the ability of endophytic fungi isolated from four different plants which are celery, parsley, mint, and coriander to inhibit the growth of phytopathogenic fungi isolated from the infected tomato. On the other hand, an in-silico screening of the antifungal activity of some fungal metabolites as inhibitors of the enzyme 3HNR by molecular docking was done in order to propose new natural and eco-friendly antifungal substances.

Materials and Methods

Isolation, purification, and identification of endophytes

In this study, four plant species were chosen for the isolation of endophytic fungi. These plants are mint, parsley, celery, and coriander. The plants were transported to the laboratory, where they were subjected to a surface sterilization procedure. Initially, all plants were washed in tap water to remove soil particles and adhered

debris. This was followed by disinfection with 95% ethanol for 30 seconds, then in 10% sodium hypochlorite for 2 minutes followed by treatment with 75% ethanol for 2 min. Finally, the plants were washed twice in sterile distilled water for 1 minute (Ezra and Strobel, 2004).

The sterilized stems, roots, and leaves of each plant are cut into small pieces (0.5 x 0.5 cm²) using a sterile scalpel. The pieces were then placed on the PDA medium. Each dish received 04 pieces of each plant tissue (stem, leaf, or root). To obtain a typical development, the inoculation was carried out in a single point by depositing the cutting tissues in the center of a Petri dish (Botton *et al.*, 1990). The Petri dishes were then incubated for 7 days at 30 °C (Ravlomanantsoa, 2004). The purification was performed on the same isolation medium and with the same incubation conditions.

Strain identification

The macroscopic characterization of fungi was based on the morphology, shape, and color of the mycelium. The identification was done by direct observation on the agar after purification. It was based especially on the following characters: (1) the speed of growth: by measuring the diameter of the colony; (2) the texture of the colony: velvety, woolly, and powdery; (3) the color of the front and back of the colony; (4) the presence or absence of a diffusible pigment in the medium; (5) the colony shape: regular, irregular, jagged, and filamentous; (6) the exudate: presence or absence of droplets.

Microscopic observation was made at magnifications x40 using the tape technique that consists in adhering a mycelial fraction from a young culture with a piece of tape and sticking it on a blade containing a few drops of methylene blue (Chabasse *et al.*, 2002).

Isolation, purification, and identification of phytopathogenic fungi

Isolation of the pathogen was performed from affected tomatoes specimens. The samples were rinsed with tap water for a few minutes to eliminate adherent debris, then disinfection of the surface is performed by successive soaking of small pieces (4 mm²) of plants (taken from the intersection of the lesion and the healthy tissue) in a 70% ethanol solution for 1min and then in 3% sodium hypochlorite for 4 min. There after, the disinfected pieces were tricked for the second time in 70% ethanol solution for 30 sec, then rinsed three successive times with sterile distilled water for 1 minute each time, and finally leave to dry in a sterile area (Kumar *et al.*, 2007).

After the surface sterilization step, the plant pieces were aseptically transferred onto PDA medium to allow pathogen emergence, this is done at a rate of one fragment in the center of each Petri dish. The plates were finely incubated at 30 °C for 4 to 7 days (Zeroug, 2011). Purification and identification were performed in the same way as those for the purification and identification steps of endophytic fungi.

Screening of antagonistic isolates

The antagonism test consists in looking for the antifungal activity of the endophytic strain against phytopathogenic fungi. To do this, a spore suspension of the pathogen of 7 days old was prepared, and then a sterile swab was dipped in this suspension to uniformly seed the surface of plates containing PDA agar. After surface drying (about 5 min), 6 mm diameter agar cylinders of pure and young isolates of endophytic fungi of 7 days old were picked up by a sterile Pasteur pipette (used as a cutter) and deposited on the PDA agar previously inoculated with the spore suspensions. Each plate received 05 agar cylinders. Petri dishes were incubated at 30 °C for 3 days. The diameters of the inhibition zones were measured (Madigan *et al.*, 1997).

In silico analysis

Preparation of the target protein's 3D structure

The 3D structure of 3HNR was obtained from the PDB database with the identifier: ID 1JA9. In order to remove solvent molecules, water, and co-crystallized ligands bound to the receptor, Chimera software was used. The structure of the prepared receptor was saved in Mol2 format.

Preparation of the ligands

For docking analysis, about 100 compounds were selected based on the literature review. All of the ligand molecules are secondary metabolites produced by different fungi strains. The canonical SMILES of these compounds were obtained from the PubChem database. They were used to design the three-dimensional structures by Chimera software. All structures were saved in Mol2 format for their use in the docking process.

Molecular docking process

To improve the result of docking and receptor-ligand interactions, the target protein and ligands were subjected to structure optimization by the addition of hydrogen atoms and energy minimization, to develop more stable structures. This step was established by Chimera software. For the docking process, all ligands were docked using the Vina program as a docking engine to find the most promising binding geometry (Forli et al., 2012). The center of the grid box was set as (X: 82.34, Y:15.37, Z: 16.38Å), while the dimension was (X: 10.00, Y:10.00, Z:9.00Å). To confirm the validity of the docking process, the operation was initially performed with the co-crystallized ligand of the 3HNR that is Phtalide. The potential molecules with the best binding affinities for the target receptor are those with binding energy lower than that of the co-crystallized molecule Phtalide.

To visualize and study the interaction modes between the ligands and the receptor, the docked conformations were analyzed using Discovery Studio software to identify the types of bonds between the amino acid residues of the receptor and the ligands.

ADMET and drug-likeness analysis

The pharmacokinetic study was established by the SwissAdme and ADMETSAR programs to characterize the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of selected ligands. This study included different factors, such as carcinogenicity, mutagenicity, cardiotoxicity, hepatotoxicity, bioavailability, and synthetic accessibility (Cheng *et al.*, 2012).

Results and Discussion

In recent years, there has been an increasing demand for safe and environmentally friendly agricultural products (Huh and Kim, 2010). As a result, biological control practices are in much greater demand as alternatives to synthetic pesticides. Biological control practices are particularly important in organic crop production and disease control (Cook, 1993; Yang *et al.*, 2002; Kim and Yun, 2011).

Identification of endophytic fungi

In this study, seven endophytic fungal strains were isolated from the different plant tissues. From the 07 strains, 04 were isolated from celery, 02 from mint, and only one from parsley, but none from coriander.

The micro and macroscopic characters of the different fungal isolates were studied on a PDA medium. The identification is based on the appearance of the colonies (consistency, color of the surface, and reverse side of the colony) as well as the microscopic characters (shape and arrangement of conidia, conidiophores, and septation of the mycelium).

For the isolate Ena, an olive black colony with dark pigment formed in concentric rings towards the edge of the Petri dish after 7 days of incubation. Multicellular conidia formed on a distinctive conidiophore with a darker terminal bulge, hyphae are septate (Figure1). These results showed that this genus belongs to the genus *Stemphylium* (Kidd *et al.*, 2016).



Figure 1. Result of macro and microscopic observation of the endophytic isolate ENa; (A) macroscopic characterization; (B) microscopic characterization

The isolate ENb showed a clear pink colony with aerial mycelium formed in concentric rings towards the edge of the Petri dish after 7 days of incubation. The conidiophores are loosely branched and the hyphae are septate (Figure 2). These results show that this strain belongs to the genus *Fusarium* (Kidd *et al.*, 2016).

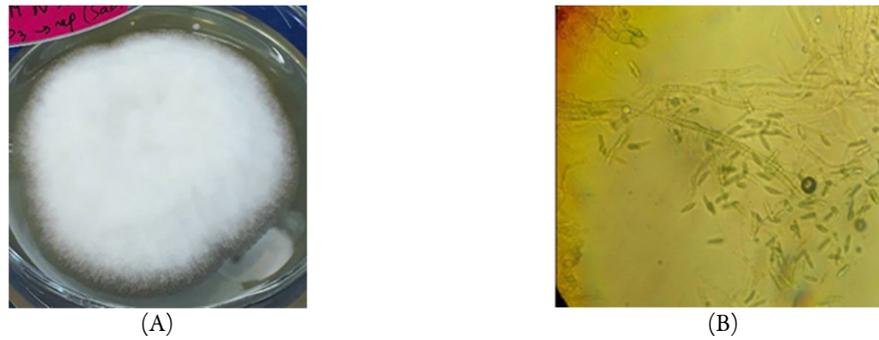


Figure 2. Result of macro and microscopic observation of endophytic isolate ENb; (A) macroscopic characterization; (B) microscopic characterization

Concerning the three isolates ENd, ENf, and ENg the colonies are black to olive or Greyish formed in concentric rings towards the edge of the Petri dish after 7 days of incubation (Figure 3). Microscopic observation shows branched and acropetal chains, multicellular conidia, and septate hyphae. These characters attribute the 3 isolates ENd, ENf, and ENg to the genus *Alternaria* (Kidd *et al.*, 2016).

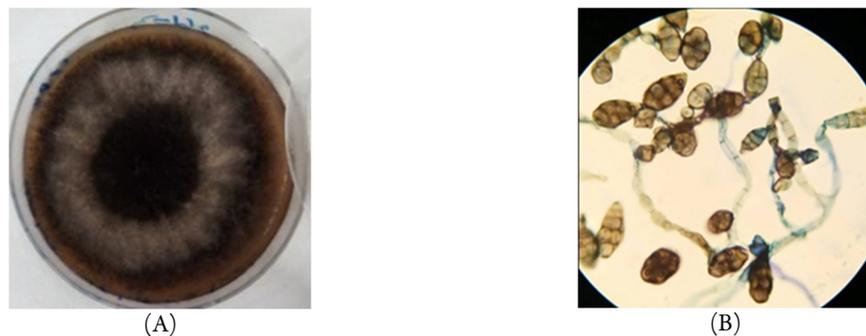


Figure 3. Result of macro and microscopic observation of endophytic isolates ENd, ENf, and ENg; (A) macroscopic characterization; (B) microscopic characterization

A green colony formed in concentric rings towards the edge was observed for the isolate ENo. Microscopic observation showed hyphae septate with branched chains of globular spore. The dark conidiophores are simple or branched with chains of conidia (Figure 4). Based on these results, this strain can be assigned to the genus *Trichoderma* (Kidd *et al.*, 2016).

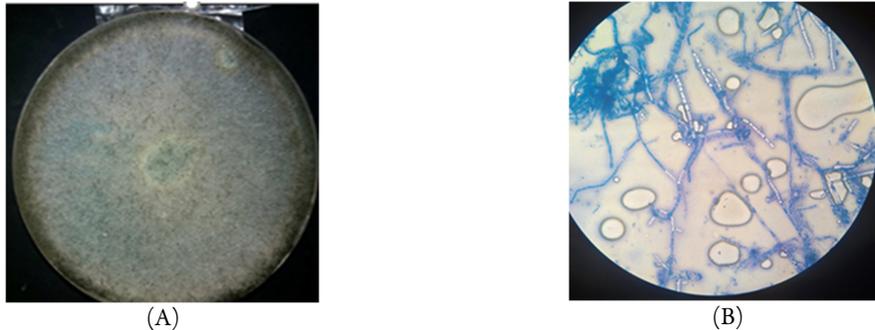


Figure 4. Result of macro and microscopic observation of endophytic isolates ENo; (A)macroscopic characterization; (B)microscopic characterization

Identification of phytopathogenic fungi

The isolates Tom1 and Tom3 showed colonies of green-black color formed in concentric rings of the Petri dish after 7 days of incubation (Figure 5). Microscopic observation showed branched acropetal chains, conidia multicellular, and septate hyphae. These results show that Tom3 belongs to the genus *Alternaria* (Kidd *et al.*, 2016).

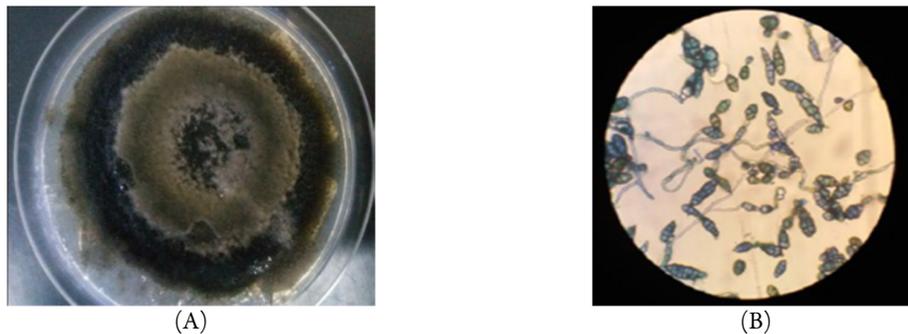


Figure 5. Result of macro and microscopic observation of pathogenic isolates Tom1 and Tom3; (A) macroscopic characterization; (B) microscopic characterization

Concerning the isolate Tom 6 and Tom 10, a white colony in the periphery with a green-blue center and red diffusible pigment was observed in the PDA medium (Figure 6). For the microscopic observation, septate hyphae and conidiophore were remarked. The phialides were grouped in brush round conidia. These results indicate that this strain probably belongs to the genus *Penicillium* (Kidd *et al.*, 2016).

The 02 isolates Tom1 and Tom3 were identified as *Alternaria*. Tom6and Tom 10 were identified as *Penicillium*, respectively. According to our results, isolates belonging to the genus *Alternaria* are the most represented among all the strains of phytopathogenic fungi isolated from tomatoes. This result was quite expected due to the important place of this taxon as phytopathogenic agents of several types of plants.

Several species of the genus *Alternaria* have a necrotrophic saprophytic lifestyle that causes real diseases to the host plants. The symptoms of *Alternaria* are generally manifested by hazy to blackish spots on leaves, stems, and fruits (Rodrigous *et al.*, 2010).



Figure 6. Result of macro and microscopic observation of pathogenic isolate Tom10; (A) macroscopic characterization; (B) microscopic characterization

The ubiquitous fungus *Penicillium* is the dominant postharvest pathogen among fruits and vegetables, primarily pome fruits. Due to its ability to grow at low temperatures, *Penicillium* has also been associated with widespread fruit deterioration during storage. In addition to the aesthetic aspect of its presence, *Penicillium* contamination poses a health hazard due to the production of toxic secondary metabolites (mycotoxins) in contaminated fruit. Among the mycotoxins produced by *Penicillium*, polyketide patulin is the most notable contaminant, given its long-established toxicity and prevalence in fruit infection (Tannous *et al.*, 2020).

Screening of the endophyte's antagonistic activity against phytopathogenic fungi

The demonstration of the antifungal activity of endophytic isolates consists of the search for their antagonistic effect on the development of the phytopathogenic fungi isolates. This study was done *in vitro* using the agar cylinder method. Antagonisms results are obtained after 72 hours of incubation and are manifested by the absence or presence of a zone of inhibition more or less different according to the antagonists. Table 1 represents the inhibition zone measured in mm.

Table 1. Inhibition zone diameters (mm) of the pathogen isolates by the endophytic strains

| Pathogenic isolates | Endophytic isolates | | | | | | |
|---------------------|---------------------|-------|-----|------|------|-----|------|
| | ENa | ENb | ENd | ENf | ENg | ENs | ENo |
| Tom1 | - | 20 mm | - | - | - | - | - |
| Tom 3 | 10mm | 20mm | - | - | 20mm | - | 25mm |
| Tom 6 | - | 20 mm | - | 20mm | - | - | - |
| Tom 10 | 23mm | 23 mm | - | 40mm | 20mm | - | 30mm |

-: no inhibition.

According to the results obtained, the isolate ENb attributed to the genus *Fusarium* and ENo identified as *Trichoderma* gave a significant antifungal effect against strain Tom10 (*Penicillium* sp.) with 23 and 30 mm of inhibition zone, respectively, and Tom3 (*Alternaria*) with 20 and 25 mm of the inhibition zone, respectively. Based on these results, the endophytic strains ENb and ENo appear to have strong antifungal activity against the phytopathogens *Penicillium* and *Alternaria*, making them important strains due to the remarkable antagonistic abilities that make them promising strains in a biological control strategy.

The results obtained by the *in vitro* antagonism test showed that the tested endophytic strains produce antifungal metabolites, and the fact that the strains gave different diameters of inhibition can be explained by different modes of action and different types of biocontrol mechanisms. These isolates can have many mechanisms of action that include them in integrated disease management programs. They can also be used as biocontrol agents. Secretion of inhibitory metabolite produced by the antagonist (antibiosis), extracellular hydrolytic enzymes, competition for space and nutrients between organisms, and detoxification of virulence factors are mechanisms involved in biological disease control (Thambugala *et al.*, 2020).

Fusarium is a worldwide fungal species complex that lives in the soil and rhizosphere and comprises both pathogenic and nonpathogenic strains (Leslie and Summerell, 2006). Sajeena *et al.* (2020) showed that non-pathogenic *Fusarium* is a promising group of endophytic fungi with antagonistic potential that can boost host plant growth. On the other hand, the species of the genus *Trichoderma* are known for their production of antibiotics affecting other microorganisms but also for their ability to act as biocontrol organisms (Weindling, 1934; Weindling and Fawcett, 1936).

Species of *Trichoderma* are known to deploy several mechanisms to keep its opponents in check as mycoparasitism reaction, where the antagonist attacks the pathogen by piercing the hyphae and invading them. It was demonstrated that *Trichoderma* enters the hyphae of *Rhizoctonia solani* by lysis of the walls using the enzymes glucanase and chitinase (Elad *et al.*, 1983). On the other hand, *Trichoderma* exerts a fungistatic action at a distance (volatile antibiotics) mainly affecting young hyphae. This is especially due to *Trichoderma viride*, while another species *Trichoderma harzianum* inhibits the formation of sclerotia in *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Corbaz, 1990).

In silico analysis

Molecular docking

Molecular docking calculations were performed using AutoDockVina, where the top four candidate ligands were selected based on their binding affinity. The compound Phthalide, the co-crystallized ligand, had an energy of -5.3 Kcal/mol in the docking study and showed hydrophobic interaction with Tyr208(4.03Å°) and electrostatic interaction with Met200(5.69Å°). On the other hand, the fungal metabolite Phenylethyl alcohol gave binding energy of -5.2Kcal/Mol and three different interactions, which are two hydrogens with the residues Tyr208(3.83Å°), and Ile150 (5.45Å°) and one electrostatic bond with Met267(5.98Å°). Whereas, the ligand Tyrosol showed a binding energy of -5.1Kcal/Mol and three bonds, which are one hydrogen bond with the amino acid Cys205 (2.82Å°), and one hydrophobic bonds with Tyr208(3.81Å°), and one electrostatic bond with Met200 (5.93Å°). The third ligand p-Hydroxybenzaldehyde had binding energy of -5.0Kcal/Mol and four bonds which are two hydrogen bonds with Tyr163 (2.49Å°), Ser149 (3.49Å°), and one hydrophobic bonds with Tyr208(3.83Å°), and one electrostatic bond with Met200 (5.89Å°) (Table 2, Figure 7 and 8).

Table 2. Result of molecular docking and type of bonds between the three top-ligands and 3HNR receptor

| Ligands | Score (Kcal/mol) | Hydrogen interactions | Hydrophobic interactions | Electrostatic interactions |
|-----------------------|------------------|--|----------------------------------|----------------------------|
| Phthalide | -5.3 | none | Tyr208(4.03Å°) | Met200(5.69Å°) |
| Phenylethylalcohol | -5.2 | none | Tyr208(3.83Å°) Ile150(5.45Å°) | Met267(5.98Å°) |
| p-Hydroxybenzaldehyde | -5.0 | O1-Tyr163(2.49Å°), O1-Ser149 (3.49Å°) | Tyr208(3.83Å°) | Met200(5.89Å°) |
| Tyrosol | -5.1 | O2-Cys205 (2.82Å°) | Tyr208(3.81Å°) | Met200(5.93Å°) |

ADMET and drug-likeness analysis

SwissADME and ADMETSar give detail on the physicochemical profile and the medicinal chemistry property of the top three fungal metabolites. The obtained results are summarized in Tables 3 and 4.

The partition coefficient and solubility are two physicochemical parameters that are considered to play important roles in the efficiency of medicament candidates. Based on the predicted LogP value, it is concluded that the studied compounds lie within the interval value of 1.10 to 1.64 which is less than 5. In addition, the four molecules respect the majority of the roles of Lipinski, Veber, and Ghose, where all the derivatives satisfied the rule, ($MW \leq 500$ Da, $\text{LogP} < 5$, $\text{nHBD} \leq 5$, $\text{nHBA} \leq 10$, and $\text{TPSA} < 140$ (Å²)).

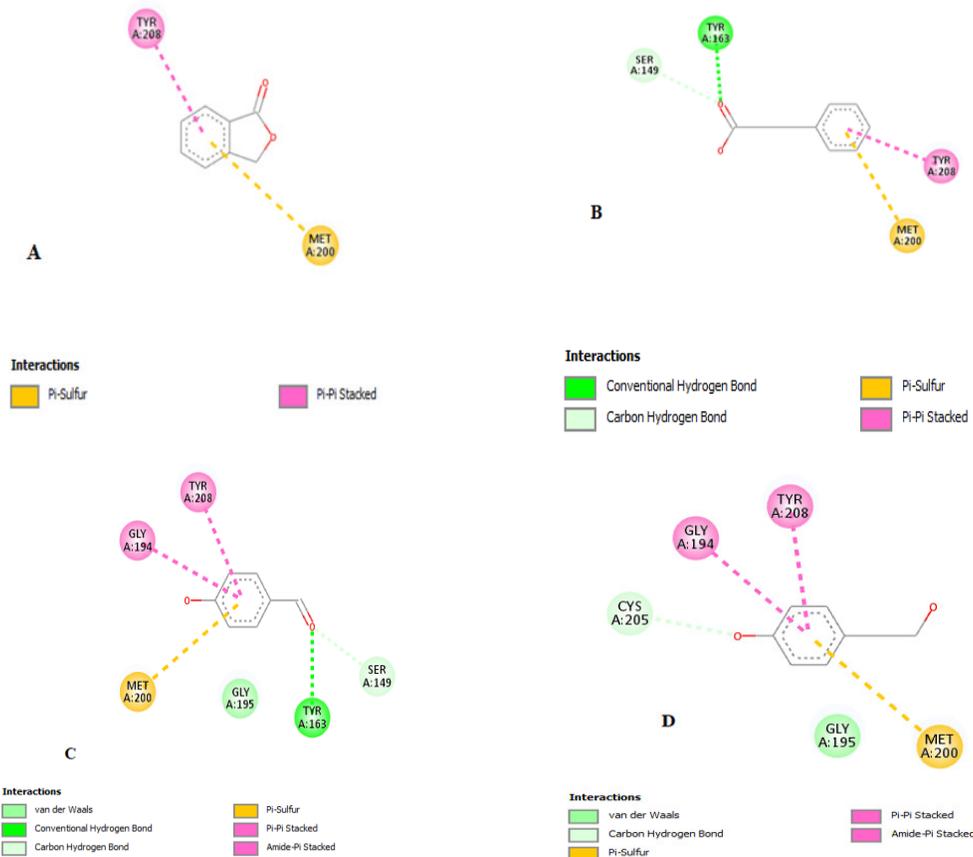


Figure 7. 2D structures of 3THNR receptor and the studied ligands; (A): Phthalide; (B): Phenylethyl alcohol; (C): p-Hydroxybenzaldehyde; (D): Tyrosol

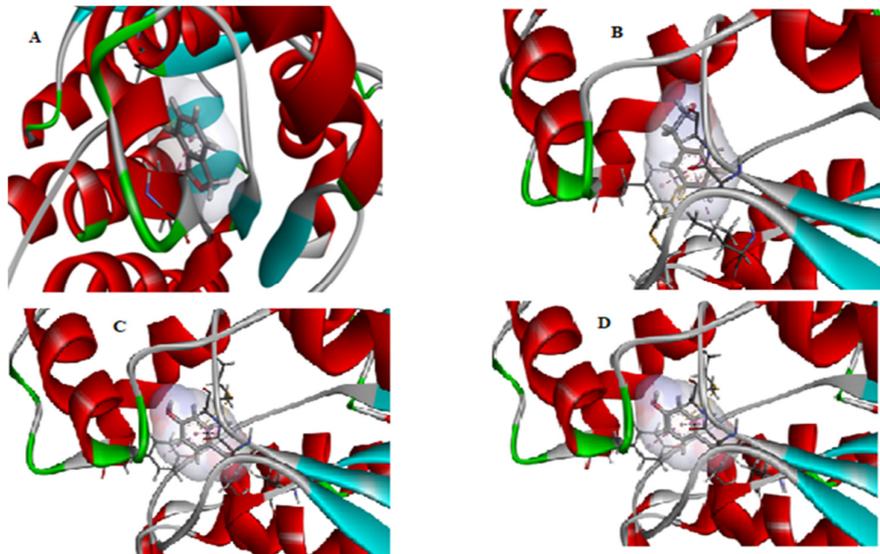


Figure 8. 3D structures of the interactions between the 3HNR receptor and the studied ligands; (A): Phthalide; (B): p-Hydroxybenzaldehyde; (C): Tyrosol; (D): Phenylethyl alcohol

Table 3. Result of drug-likeness of the top ligands

| Ligand | MW (g/mol) | HBD | HBA | TPSA (Å ²) | Log P | Log S | Lipinski | Ghose | Veber | BBB permeability |
|-----------------------|------------|-----|-----|------------------------|-------|-------|----------|-------|-------|------------------|
| Phthalide | 134.13 | 2 | 0 | 26.30 | 1.46 | -2.54 | yes | no | yes | yes |
| Phenylethylalcohol | 122.16 | 1 | 1 | 20.23 | 1.64 | -2.58 | yes | no | yes | yes |
| p-Hydroxybenzaldehyde | 122.12 | 2 | 1 | 37.30 | 1.17 | -1.72 | yes | no | yes | yes |
| Tyrosol | 138.16 | 2 | 2 | 40.46 | 1.10 | -2.03 | yes | no | yes | yes |

MW: molecular weight; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; TPSA: topological polar surface area; BBB permeability: blood-brain barrier permeability

Table 4. Result of ADMET of top ligands

| Ligand | Caco2 | HIA | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | Ames test | Herg inhibition | HHT |
|-----------------------|-------|-----|------------------|-------------------|------------------|------------------|------------------|-----------|-----------------|----------|
| Phthalide | yes | yes | no | no | no | no | no | mut | negative | negative |
| Phenylethylalcohol | yes | yes | yes | no | no | no | no | nM | negative | negative |
| p-Hydroxybenzaldehyde | yes | yes | no | no | no | no | no | nM | negative | negative |
| Tyrosol | yes | yes | no | no | no | no | no | nM | negative | negative |

Caco2: permeability assay; HIA: human intestinal absorption; Herg: human ether-go-go related gene potassium channel; HHT: human hepatotoxicity; mut: mutagen; nM: non-mutagen.

ADMET analysis was also carried out to identify whether the studied compounds could lead to toxicity, mutagenicity, or metabolic instability. Table 4 showed that except for Phenylethyl alcohol which inhibits cytochrome CYP1A2, the other ligands have no inhibitory effect on all cytochrome families. In addition, all four molecules give a good intestinal absorption and oral bioavailability as shown by the BBB permeability, Caco2, and HIA measurements. AMES toxicity test was employed to know whether the studied compounds are mutagenic or not. All the ligands displayed a negative AMES toxicity test, which means that the ligands are non-mutagenic. The carcinogenic and hepatic-toxicity profile also revealed that the ligands were nontoxic and safe.

Conclusions

In this work, an *in vitro* and *in silico* study of the biofungicide potential of some fungal agents was developed. The *in vitro* part consisted of the isolation and identification of endophytic fungal strains from four plants (parsley, coriander, mint, and celery). As well as the screening of their antagonism against phytopathogenic fungal strains isolated from infected tomatoes. The results of the macroscopic and microscopic examination of the endophytic fungal strains highlighted 9 strains representing different genera, which were identified as three strains *Alternaria* sp., one strain *Fusarium* sp., one strain *Trichoderma* sp., and one strain *Stemphylium* sp. The isolation of fungal strains from infected samples of tomatoes showed 7 strains identified as *Alternaria* sp. and *Penicillium* sp.

The antagonistic effect of the endophytic fungal strains against the different phytopathogenic fungal isolates was tested firstly by the agar cylinder technique, the strains *Fusarium* sp. and *Trichoderma* sp. showed significant activity against certain phytopathogenic strains of *Penicillium* sp. and *Alternaria* sp.

In the *in-silico* study, the molecular docking of the 3HNR enzyme was done by the screening of the inhibitory effect of a number of 100 secondary metabolites produced by fungi. Only three molecules (Phenylethyl alcohol, p-Hydroxybenzaldehyde, and Tyrosol) gave significant binding energy close to the value of the co-crystallized ligand Phthalide. These three molecules interact with the 3HNR receptor with a remarkable number of bonds of different types (hydrogen, hydrophobic or electrostatic). On the other hand, the pharmacokinetic and ADMET study showed that the three promising ligands respect the majority of the ADMET and drug-Likeness rules and therefore can be considered as a good natural candidate in the biological control against the pathogenic fungal of plants.

Authors' Contributions

Both 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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