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In silico assessment of *Rhanterium adpressum* sesquiterpenes inhibitory effect on 3 and 15-*O*-trichothecene acetyltransferases

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Abstract

Essential oils (EO) from leaves and flowers of *Rhanterium adpressum* have shown to inhibit the mycelial growth and type B trichothecenes production. The four strains of *Fusarium culmorum* and *Fusarium graminearum* were inhibited with 0.25 µL.mL^{-1} of each oil. The inhibitory activity of 11 sesquiterpenes identified in these oils was here examined *in silico* against two key enzymes in the biosynthesis pathway of trichotecenes namely: 15-*O*-trichothecene acetyltransferase and 3-*O*-trichothecene acetyltransferase. In sesquiterpene composition, T-muurolol and α-eudesmol have the highest percentages ranging from 1.4 to 2.75 %. Three-dimensional structures of these two enzymes were modeled using SWISS-MODEL with GMQE = 0.93 and QMEAN = -0.45 for 3-*O*-trichothecene acetyltransferase and GMQE = 0.93, QMEAN = -0.58 for 15-*O*-trichothecene acetyltransferase. By the results of docking, T-muurolol and *α*-eudesmol showed high affinity compared to 15-decalonectrin and deoxynivalenol. These molecules are all sesquiterpenes with no major conformational difference with an RMSD of 3.7 Å and 3.5 Å between 15-decalonectrin and *α*-eudesmol, T-muurolol respectively. The results of docking prove the inhibitory effect of *R. adpressum* EO sesquiterpenes on the enzymes of mycotoxins biosynthesis pathway of *F. culmorum* and *F. graminearum*.

Introduction

Fusarium culmorum and *Fusarium graminearum* are two closely related species, known by their pathogenicity and mycotoxin production of type B trichothecenes considered major determinants of their aggressiveness. It has been reported that these strains are more common on wheat grain in Germany, Netherlands and other countries with high levels of deoxynivalenol (DON) (Waalwijk *et al.* 2003; Shah *et al.* 2005). Two chemotypes have characterized these strains according to their production of trichothecenes: the NIV chemotype, which includes isolates producing Nivalenol and Fusarenone X, and the DON chemotype, which includes isolates producing DON and acetyl-deoxynivalenol (ADON) (Bakan *et al.* 2001). Trichothecenes are a large group of chemical sesquiterpene epoxides therefore they all have in common a 12,13-epoxytrichothecene skeleton and characterized by a keto group in the C8 position and with its presence or absence can be distinguished type A trichothecenes and type B trichothecenes. Both of *F. culmorum* and *F. graminearum* produce type B trichothecenes, including deoxynivalenol (DON), nivalenol (NIV) and their several acetylated derivatives. 3-acetyl-deoxynivalenol (3ADON) sub-chemotype was

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identified in *F. culmorum* while *F. graminearum* displayed 15-acetyl-deoxynivalenol (15ADON) sub-chemotype (Foroud and Eudes 2009; Yörük and Albayrak 2012).

The biosynthesis of *Fusarium* trichothecenes has been studied in *F. sporotrichioides*, which produces T-2 toxin. In the biosynthetic pathway, many genes are involved such as Tri 5, Tri 6, Tri 13, Tri 7, Tri 11, Tri 3, Tri 8 and Tri 16 (Garvey *et al*. 2009). The core structure of trichothecenes is formed from the cyclization of farnesyl pyrophosphate by trichodiene synthase, then the modifications of positions C4 and C15 are carried out by P450 monooxygenase/acetyltransferase followed by oxygenation of C8. Finally, to protect itself, the fungus removes the acetyl group at C3 who added it at the beginning of the biosynthesis pathway (Wagacha and Muthomi 2007; Yörük and Albayrak 2012).

In a previous study, the essential oils of the leaves and flowers of *Rhanterium adpressum* showed remarkable effects on the mycelial growth of four strains of *F. culmorum* and *F. graminearum* (T5, BD17, INRA 349 and INRA 812). This effect was accompanied by a strong inhibition of type B trichothecenes production with 0.25 $\mu L.mL^{-1}$ of each oil, adding that it has a variability in the chemical composition between the two oils that has influenced the biological activity. In this study, *in silico* modeling was performed to verify the inhibitory effect on the production of type B trichothecenes (sesquiterpene secondary metabolites) of sesquiterpenes present in the oil of *R. adpressum* on two key enzymes in mycotoxins biosynthesis in both strains: 15-*O*-trichothecene acetyltransferase (*F. graminearum*) and 3-*O*-trichothecene acetyltransferase (*F. culmorum*).

Experimental

Plant material, extraction and identification

Collection of aerial parts of *Rhanterium adpressum* was done in three months from each year: 2011, 2012 and 2013 in Zelfana 660 km SSE of Algiers (32°23’46” N; 5°13’34” E). Extraction of essential oils from dried aerial parts was performed with hydrodistillation using Clevenger apparatus for 7 h. GC/MS analysis and identification of essential oils components were described in a previous study (Elhouiti *et al.* 2017).

Homology modeling

Sequences of 15-*O*-trichothecene acetyltransferase from *Fusarium graminearum* (PCD27416.1) and 3-*O*-trichothecene acetyltransferase from *F. culmorum* (ANO39638.1) were downloaded from NCBI protein database; the number of amino acids for each protein is 517 and 511 respectively. Enzymes structures were modeled with SWISS-MODEL Workspace (Biasini *et al.* 2014) based on the target-template alignment using ProMod3 Version 1.1.0. In SWISS-MODEL, the modeling process comprises four steps, beginning from the identification of structural template(s), alignment of target sequence and template structure(s), model building and model quality evaluation where the best homology models were selected according to Global Model Quality Estimation (GMQE) and Qualitative Model Energy Analysis (QMEAN) statistical parameters (Benkert *et al.* 2009; Biasini *et al.* 2014). For flexible structure comparison, secondary structures of generated enzymes models were aligned with FATCAT server (Ye and Godzik 2004).

Molecular docking

Structures of 11 sesquiterpenes from the essential oil of *R. adpressum* and structures of Deoxynivalenol and 15-decalonectrin were downloaded from PubChem database and converted into PDB files with Discovery Studio 3.5. Modeled structures of 15 and 3-*O*-trichothecene acetyltransferase and ligands were imported to Molegro Virtual Docker 6.0 (MVD). Proper bonds, bond orders, hybridization and charges were assigned to imported molecules in preparation process. The score function used is MolDock score with a resolution of 0.30 Å and the coordinates of the search space are: X: 8.56, Y: 32.65, Z: 25.51 at 10 Å. The selected search algorithm is MolDock optimizer 10 runs with optimization of the energy and optimization of H-bonds at the end of the docking. The other parameters for this evolution algorithm are: population size = 50, maximum interactions = 2000, scaling factor = 0.5, cross over rate = 0.9

Table 1. Percentages of sesquiterpenes in the essential oil of *R. adpressum*.

	2011			2012		
	April	May	June	April	May	June
	Peak Area [%]			Peak Area [%]		
α-Humulene	0.48	0.37	0.41	0.46	0.41	0.12
Germacrene D	0.32	0.37	0.46	0.31	0.26	0.55
Bicyclogermacrene	0.51	0.66	1.24	2.08	1.25	0.05
β-Bisabolene	0.20	0.24	0.33	0.13	0.15	0.19
γ-Cadinene	0.49	0.55	0.54	0.47	0.41	0.34
δ-Cadinene	1.03	0.96	0.63	0.61	0.85	0.07
γ-Selinene	0.82	0.87	0.19	0.85	0.93	0.21
Nerolidol	1.07	1.06	1.19	0.96	1.25	1.68
Ledol	0.79	0.49	0.54	0.50	0.51	0.11
T-Muurolol	2.41	2.31	2.75	2.27	1.40	2.12
α-Eudesmol	2.47	1.96	2.26	2.06	2.02	1.42

and RMSD-based for termination scheme.

Results and Discussion

Sesquiterpenes in R. adpressum *essential oil*

Sesquiterpenes are less volatile than other terpenes and have great potency for stoichiometric diversity and a strong odor (Buckle 2015). In the total composition of the essential oil of *R. adpressum*, 36 components were identified represent 77 – 82 % of the total composition of which 11 sesquiterpenes belongs to hydrocarbons and oxygenated sesquiterpenes families represent 12 – 16 % of this composition (Table 1). T-muurolol and *α*-eudesmol have the highest percentages ranging from 1.4 to 2.75 %.

Important biological activities have been reported for these sesquiterpenes. These include anti-inflammatory effect of *α*-humulene in the essential oil from *Cordia verbenacea* (Fernandes *et al.* 2007), fungitoxic activity of Bicyclogermacrene (Bohlmann and Zdero 1978; Silva *et al*. 2007), antibacterial activities, antileishmanial and antiulcerogenic of Nerolidol (Hada *et al*. 2003; Arruda *et al*. 2005; Klopell *et al*. 2007), antibacterial activity of *β*-bisabolene (Nascimento *et al*. 2007) and antitermitic activity of T-muurolol (Cheng *et al*. 2004).

In a previous study, variability between chemical groups and in the same group was noted, which may be related to the variation of different biotope conditions (Elhouiti *et al.* 2017). Yayi-Ladekan *et al*. (2012) observed a diurnal variation in the chemical composition of the essential oil of *Ocimum canum* which modified the synergistic action of these constituents in the results of the antimicrobial activity. These sesquiterpenes identified in the essential oil of *R. adpressum* have been suggested as molecules which may be involved in mycotoxins production inhibition of the strains of *F. culmorum* and *F. graminearum* observed in the previous study (Elhouiti *et al.* 2017). The hypothesis was that their structural analogy with trichothecenes may play an important role in inhibiting the biosynthetic enzymes of these mycotoxins such as 15-*O*-trichothecene acetyltransferase and 3-*O*-trichothecene acetyltransferase.

Homology modeling

Modeling of both structures of 15-*O*-trichothecene and 3-*O*-trichothecene acetyltransferase with ProMod3 1.1.0 of SWISS-MODEL gave one model for 3-*O*-trichothecene acetyltransferase with GMQE = 0.93 and QMEAN = -0.45 (86.06 % of sequence identity to 3fot.1.A sequence template) and two models for 15-*O*-trichothecene acetyltransferase: the first one has 85.43 % of sequence identity to 3fot.1. A sequence template and GMQE = 0.93, QMEAN = -0.58. The sequence identity to the sequence template (1noc.2.A) for the second model was 12.64 % with GMQE = 0.14, QMEAN = -6.64. In order to select the most suitable templates, the most likely structural similarity is the value at which the joint distribution is maximized, called global quality estimation score (GMQE) expressed as a number between zero and one, where higher numbers indicate higher

Table 2. Docking analysis of *R. adpressum* sesquiterpenes, deoxynivalenol and 15-decalonectrin on 3 and 15-*O*-trichothecene acetyltransferases.

	3-*O*-trichothecene acetyltransferase						**15-*O*-trichothecene acetyltransferase**					
	MolDock Score	**Rerank Score**	**Internal**	**HBond**	**LE1**	**LE3**	**MolDock Score**	**Rerank Score**	**Internal**	**HBond**	**LE1**	**LE3**
α-Humulene	-71.5	-57.6	6.2	0	0.4	0.6	-76.2	-30.1	6.2	0	0.4	0.6
Germacrene D	-90	-69.4	-5.4	0	-0.4	0.1	-78.1	-62.4	-5.2	0	-0.3	0.1
Bicyclogermacrene	-70.8	-34.3	0.3	0	0	0.3	-76.9	19.1	0.3	0	0	0.3
β-Bisabolene	-93.4	-77.1	8.3	0	0.6	0.6	-86.4	-72.9	5.7	0	0.4	0.5
γ-Cadinene	-79.2	-63.2	7	0	0.5	0.5	-67.8	-56.3	6.2	0	0.4	0.5
δ-Cadinene	-70.5	-47.9	12.7	0	0.8	0.9	-59.6	-50.2	11.6	0	0.8	0.8
γ-Selinene	-68.9	-60.1	13.4	0	0.9	0.6	-75.5	-63.3	13.4	0	0.9	0.6
Nerolidol	-103.9	-88.8	9.5	-2	0.6	0.5	-91.5	-80.2	4.3	-2.4	0.3	0.2
Ledol	-73.5	-60.5	-0.5	-1.9	0	0.2	-76.6	-62.4	-0.5	-1.3	0	0.2
T-Muurolol	-67	-59.7	14.6	-2.5	0.9	0.7	-63.3	-55.9	12.7	-0.2	0.8	0.6
α-Eudesmol	-76.3	-64.2	17.5	-2.5	1.1	0.8	-77.5	-69.4	14.4	-2.2	0.9	0.6
Deoxynivalenol	-81	-68.1	31.8	-12.3	1.5	0.7	-69.2	-0.4	37.9	-8.8	1.8	0.7
15-Decalonectrin	-79.4	-19.9	18.6	-5.9	0.8	0.5	-72.5	-66.4	22.2	-2.5	1	0.6

reliability (Biasini *et al*. 2014). Global model quality is assessed with the local composite scoring function QMEAN calculated as indicators for the overall model quality and takes values between 0 and 1 (Benkert *et al*. 2009; Biasini *et al*. 2014).

With the structural alignment of FATCAT, the two structures of 15-*O*-trichothecene and 3-*O*-trichothecene acetyltransferase are significantly similar ($P \leq 0.05$) and the alignment has 496 equivalence positions with an RMSD of 0.21 Å. The alignment also showed no major difference with the template structure (3fot) with an RMSD of 0.22 Å and 495 to 489 equivalent positions for 15-*O*-trichothecene and 3-*O*-trichothecene acetyltransferase respectively.

The structures of these two enzymes belong to a conserved protein domain family (pfam 07428), which represents a conserved region of approximately 400 residues. Garvey *et al*. (2009) mentioned that in the structure of Tri 3 from *F. sporotrichioides* (3fot) there are two protein domains: N-terminal and C-terminal forming between them at doughnut hole where the active site is located. 15-*O*-trichothecene and 3-*O*-trichothecene acetyltransferase belong to the BAHD superfamily acyltransferases which have a conserved sequence catalytic motif: HX_3D and an important structural motif: DFGWG (D'Auria 2006). In both enzymes (15 and 3-*O*-trichothecene acetyltransferase), the catalytic motif is represented by: His 183, Leu 184, Phe 185, Trp 186 and Asp 187. The residues that take place of the DFGWG motif in the C-terminal are: Glu 447 - Ser 459.

Molecular docking

In docking analysis, the affinity of the *R. adpressum* sesquiterpenes was elucidated in comparison with deoxynivalenol (Tri 3 produced toxin) and 15-decalonectrin which is the native Tri 3 substrate. Binding ability of these sesquiterpenes to enzyme active site is given in terms of MolDock Score and Rerank Score (Table 2). These two scores reflect the affinity of a ligand to the active site, therefore high values indicate a strong affinity, given that Rerank score in MVD provides an estimation of interaction strength (Heble *et al*. 2016). Table 2 shows the best poses for each ligand among 65 resulting poses with their internal energies. LE1 represents ligand efficiency 1, which is the ratio between MolDock score and the number of heavy atoms while LE3 is the ligand efficiency 3 results from the division of Rerank score on the number of heavy atoms.

R. adpressum sesquiterpenes have a strong affinity for the active site of the two enzymes 15-*O*-trichothecene acetyltransferase and 3-*O*-trichothecene acetyltransferase and the oxygenated sesquiterpenes can establish hydrogen bonds with the catalytic amino acids which makes their affinities greater than the sesquiterpene hydrocarbons (Table 2). T-muurolol and α-eudesmol are the best inhibitors of these two enzymes compared to the native substrate 15-decalonectrin (Fig. 1) knowing that most of the amino acid interactions of the active site with the substrate are hydrophobic (Garvey *et al*. 2009). There is a slight structural difference between these

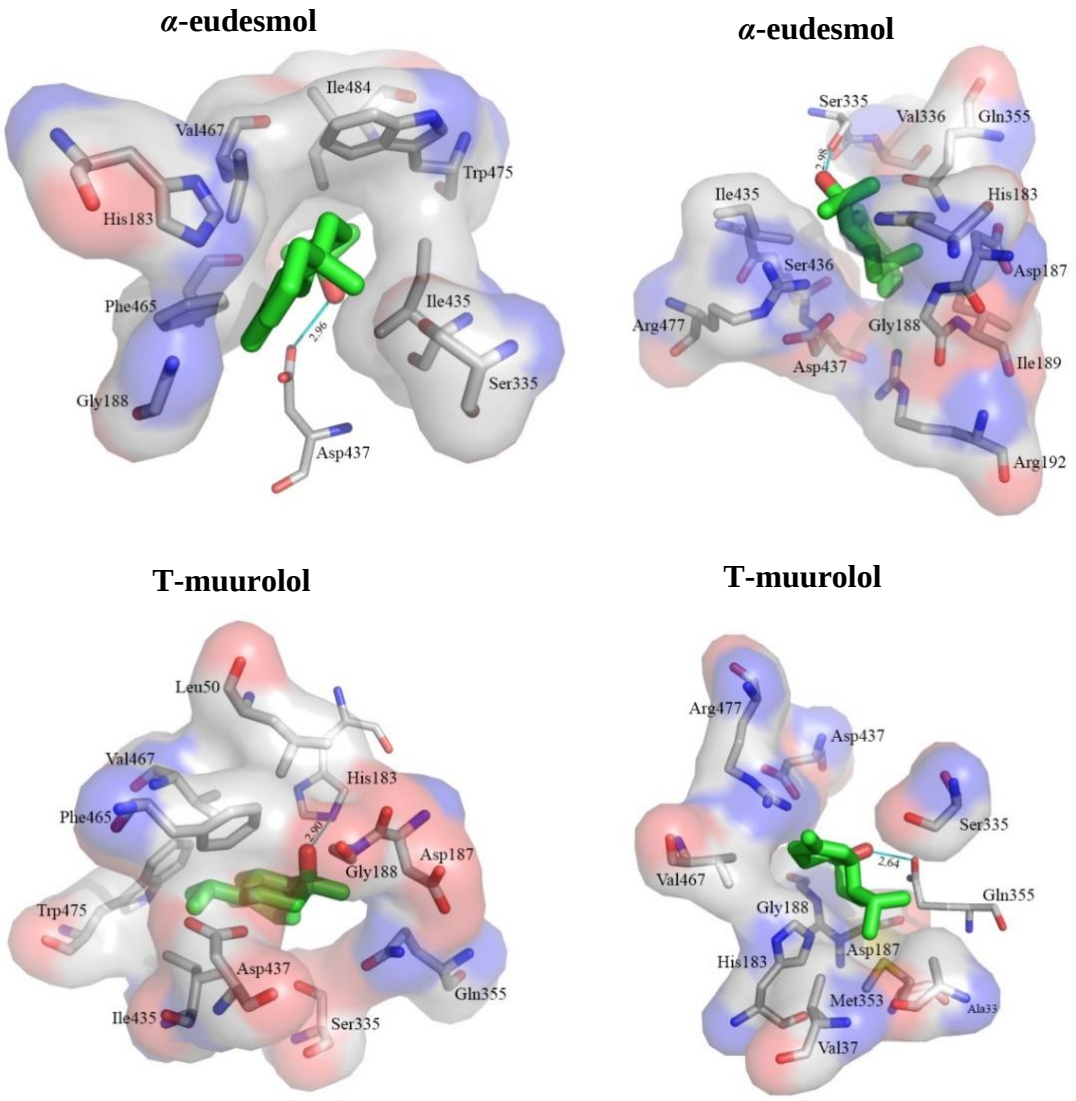

Fig. 1. T-muurolol and *α*-eudesmol docking poses in active site of 15-*O*-trichothecene acetyltransferase (on the right) and 3-*O*-trichothecene acetyltransferase (on the left).

two compounds and 15-decalonectrin with an RMSD of 3.7 Å and 3.5 Å for *α*-eudesmol and T-muurolol respectively. The analogy of these sesquiterpenes with the substrate explains the values of docking score more or less similar (Table 2) and the high affinities to catalytic active sites of the two enzymes which can make these molecules competitive inhibitors.

In catalytic process, His183 may not form a hydrogen bond with the C15 hydroxyl moiety, but does form a hydrogen bond with a trapped water molecule and the residues of the conserved loop of Lys 421 - Pro 432 are proposed to allow access to the active site. Asp 187 residue of the HX_3D motif is directed away from the substrate, forming a salt bridge with Arg 339 and participating in α-helix capping (Garvey *et al.* 2009).

In their study, Umesh *et al.* (2020), showed molecular details on the high affinity of rutin, carpaine, stigmasterol, *β*-caryophyllene, and *α*-eudesmol against trehalose-6-phosphate phosphatase (an enzyme involved in trehalose biosynthesis in the pathway of infection and proliferation of several pathogenic microbes) with distances to active site amino acids between 1.7 and 2.7 Å. In the study of Bernal and Coy-Barrera (2014), agarofuran was shown to be a very good inhibitor for *N*-myristoyl transferase, a target enzyme against protozoan parasitic diseases. On the other hand, the study of Ogungbe and Setzer (2008), showed that the monoterpene hydrocarbons (camphene, *p*-cymene, limonene, myrcene, *α*-pinene, and *β*-pinene) have a weak inhibitory

activity against cysteine protease cruzain, an enzyme that plays an important role in the penetration and proliferation of the parasite responsible for American trypanosomiasis, while the sesquiterpene hydrocarbons (*β*-caryophyllene, *α*-vopaene, germacrene D, *α*-humulene and *α*-zingiberene) showed relatively strong inhibition.

Conclusions

Essential oils of *R. adpressum* have shown a significant inhibitory effect on the growth and production of type B trichothecenes of *F. culmorum* and *F. graminearum* strains. In this study, it was shown that the sesquiterpenes and especially the oxygenated sesquiterpenes identified in *R. adpressum* oil may be responsible for the inhibition of mycotoxins production of these strains by their high affinities to biosynthesis enzymes like 15-*O*-trichothecene acetyltransferase and 3-*O*-trichothecene acetyltransferase. The results open up new perspectives in the study of the levels and mechanisms of action of these molecules of plant origin as alternatives in the fight against phytopathogenic agents.

Conflict of Interest

The authors declare that they have no conflict of interest.

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