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Molecular Docking Evaluation of the Desert Truffles as Potent Antifungal Inhibitors

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Authors' contributions

This work was carried out in collaboration among all authors. Author GMAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FKAS managed the analyses of the study. Author MURP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The research investigated the possible antifungal behavior of forty-four truffles bioactive compounds conducted to investigate the interaction modes of these inhibitors against three different types of the fungal proteins: *Candida albicans*, *Blastomyces dermatitidis*, and *Ganoderma microsporum*. The applied method in contrast to ketoconazole and griseofulvin revealed the possible anti-fungal agents ergosterol, Catechin gallate and rutin. With respect to *Candida Albicans*, the maximum possible binding energy was ergosterol (-11.75 Kcal/mol), followed then by catechin gallate (-11.46 Kcal/mol) then rutin (-9.90 Kcal/mol). Compared to Blastomyces, *Ganoderma microsporum* fungal protein with most negative binding energy among other components of the truffle is found to be of a relatively similar behavior for the same compounds. Ergosterol demonstrated the highest binding capacity for *dermatitidis*, while rutin scored the lowest against *Ganoderma microsporum*. The possible anti-fungal components of desert truffle have yet to be studied *in vitro* in the future.

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1. INTRODUCTION

Discovering new medicines derived from natural plants is becoming more popular nowadays. Microbiologists, phytochemists and botanists are exploring the environment for phytochemical ingredients that could be utilized in the treatment of infections [1]. In the last 3 decades only one group of new antifungal drugs has been reported in published material in addition to the already available, for clinical practice, three classes of anti-fungal drugs. Therefore, there are limited choices for the treatment of fungal diseases. There is also a substantial obstacle to the development of novel antifungal drugs [2]. In view of many similarities that exist between the fungal and the human cells, identification of the novel drug targets become even more difficult [3].

Abundant herbs have been documented to possess pharmacological potentials due to their phytoconstituents such as terpenes, glycosides, steroids, flavonoids, tannins, alkaloids, and many others. Various plant extracts have great potential to act against infectious agents and can therefore be used for medicinal purposes [4].

Medicinal mushrooms are really a rich number of bioactive, antimicrobial and immunostimulating polyphenols with therapeutic value. In contrast to standard research of higher epigenous basidiomyceteses, recent studies linked to hypogeous fungi and so-called desert truffles have been carried out.

In order to be able to infect a large variety of hosts, *Candida Albicans*can undergo several morphological transformations [3]. Hyphal formation is a key problem for diverse hosts, such as infectivity and invasion. For this reason, many environmental factors can stimulate *Candida Albicans* to makeup hyphae such as quorum sensing compounds, CO_2 and pH of an environment [5]. Exo-β-(1,3)-glucanases are a class of fungal enzymes, including that of the human pathogen *Candida Albicans*, which includes the likes of 5 glycosyl hydrolases. They exhibit both hydrolase and transferase control in cell wall morphogenesis including glucan metabolism [6].

Blastomycosis, a potential quite severe disease that usually starts with a distinctively subtle pneumonia-like disease that can advance, after 1 - 6 months, toa circulated phase that causes lesions to develop in capillary beds across the body, more commonly the skin, vital organs, central nervous system as well as bone marrow [7]. Blastomyces *dermatitidis* has always been the name introduced to the Ascomycetous fungus, *Ajellomyces dermatitidis*. Lately a second grouphas been defined in the genus Blastomyces, *B. gilchristii*, that subsumes those strains assigned to Blastomyces *dermatitidis* [8-11].

Bioinformatics is playing an ever-increasing role in the design and discovery of new therapeutic agents [12]. By applying computational techniques, a huge reduction in the cost of clinical and laboratory trials as well as the efforts and time needed is achieved. Rather than facilitating and accelerating the process of discovering new drug candidates, *in silico* studies can predict the pharmacokinetic properties and even the side effects [13]. Molecular docking is a computational technique that is used to predict and study the interaction mode between small molecules (ligands) and their target e.g., protein [14]. Two main steps and different types of algorithms are involved in the molecular docking protocol [15]. The first step is the generation of all possible conformations of the ligand/protein complex and the second step is measuring the binding energy of the complex using scoring functions [16]. Several docking algorithms are available such as Auto Dock, CDOCKER, Genetic Optimization for Ligand Docking (GOLD), FlexX and Glide [17]. This study aimed to show the proposed in silico anti-fungal efficacy and the mechanism of binding of 44 truffles ingredient compounds potential antifungal targets using Auto Dock 4.2.6 software.

2. MATERIALS AND METHODS

2.1 Computational Materials

Perkin Elmer Chem3D 17.1 software was used to the energy of chosen compounds [17]. Auto Dock Tools (ADT) was used to prepare the proteins and the compounds files for the molecular docking. AutoDock4.2.6algorithm was used for the docking of the proposed compounds and further prediction of the binding mode inside the active site and to estimate their binding affinity [18]. Biovia discovery studio visualizer 2019 from BIOVIA Software Inc [19].

2.2 Computational Methods

2.2.1 Preparation of the proposed compounds

Forty-four phytoconstituents ingredients of truffles were selected from the published material available on Pub Chem and saved as SDF format. The compounds were subjected to energy minimization using MM2 force field embedded in PerkinElmer Chem3D 17.1 and saved as PDB files. Then, each compound was prepared for the molecular docking by assigning the torsions and Gasteiger charges using Auto Dock 4.2.6 software and saved as PDBQT file.

2.2.2 Preparation of the proteins

The 3D structures of the *Candida Albicans* (3N9K and 3O6A) [20], Blastomyces *dermatitidis* (6C85 and 6C8W) [1], and *Ganoderma microsporum* (3KCW and 4ZIC) [21] were retrieved directly from Protein Data Bank (https://www.rcsb.org/). Crystal structures were checked for missing loops, alternate conformations, and incomplete residues using Biovia discovery studio visualizer 2019 [19] and saved as PDB format. Finally, Auto Dock 4.2.6 software was used to add polar hydrogens and Kollman charges for the structures. Then, all proteins were saved as PDBQT file.

2.2.3 Molecular docking and scoring

In this study, we used Auto Dock 4.2.6 algorithm which is considered one of the most successful and cited docking tools [22]. The grid maps for the proteins were constructed as shown in Table 1. Molecular docking was conducted using Auto Dock 4.2.6. by applying Lamarckian Genetic Algorithm (LGA). The docking parameters were kept as default except for the number of the genetics algorithm runs which was set to 150 (Docking run).

3. RESULTS

For many years desert truffles have been used traditionally as a source of nutrients and to prevent a variety of diseases such as eye diseases and skin lesions. They are rich in various chemical constituents including proteins, amino acids, fatty acids, sterols and terpenes. In the current study, in silico techniques were implemented to investigate the possible activity of a panel of truffles ingredients as potential antifungal agents. Molecular docking using AutoDock algorithm was used to study the possible binding interaction of 44 compounds from truffles against the fungal proteins of *Candida Albicans*, Blastomyces *dermatitidis*, and *Ganoderma microsporum*. Docking simulation utilizes a grid-based approach for energy evaluation, in which pre-calculated interaction energies are used as lookup tables to enable rapid evaluation of ligand-protein interactions. However, the implementation of this grid-based approach would involve rigid processing of the target molecule unless particular side chains are specifically handled outside the grid. The docked structures of ergosterol, catechin gallate, and rutin in with the *Candida Albicans* proteins are represented in Fig. 1A and B. The grid maps for the proteins were constructed as shown in Table 1.

Ketoconazole and Griseofulvin, well-known antifungal agents, were included in the study as a positive control [23]. The free binding energy scores are represented in Table 2.

Table 1. Docking grid box parameters

PDB code	Grid points dimension			Spacing (Å)	Grid center coordinate		
	X	Y	Z	0.375	X	Y	Z
3N9K	60	60	60	0.375	-3.1917	-7.5161	10.9316
3O6A	60	60	60	0.375	3.2975	-7.4503	-10.7674
6C85	60	60	60	0.375	44.2248	51.4724	460.7058
6C8W	60	60	60	0.375	-9.5609	-40.7154	14.6294
3KCW	60	60	60	0.375	-5.7265	8.7878	-71.4738
4ZIC	60	60	60	0.375	-75.0792	31.1863	-12.6683

Table 2. Binding interaction values of the 44 truffle ingredients and two references compounds (Ketoconazole and Griseofulvin)

Compound	Fungal target enzymes					
	Candida Albicans		*Blastomyces dermatitidis*		*Ganoderma microsporum*	
	(PDB: 3N9K)	(PDB: 3O6A)	(PDB: 6C85)	(PDB: 6C8W)	(PDB: 3KCW)	(PDB: 4ZIC)
	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)
Ergosterol	-11.75	-11.37	-8.54	-9.42	-10.22	-12.49
Anandamide	-7.48	-7.39	-5.14	-5.63	-8.65	-11.37
Palmitic acid	-5.52	-7.39	-5.42	-5.03	-7.59	-10.92
Palmitoleic acid	-5.57	-5.07	-5.45	-5.19	-7.59	-10.93
Heptadecanoic acid	-5.83	-4.43	-5.36	-4.75	-7.54	-11.56
Stearic acid	-5.63	-4.85	-6.43	-5.27	-8.10	-11.93
Oleic acid	-5.85	-4.91	-5.61	-5.51	-7.31	-12.02
Linoleic acid	-6.17	-5.08	-5.24	-5.72	-7.65	-11.64
Linolenic acid	-6.16	-5.30	-5.95	-5.46	-7.59	-11.32
cis-11-eicosenoic acid	-5.90	-4.88	-6.07	-4.64	-8.44	-12.47
cis-11,14-eicosadienoic acid	-5.78	-5.41	-5.42	-5.03	-8.00	-11.55
Pyrogallol	-5.96	-5.13	-5.36	-4.56	-8.09	-12.36
Homogentisic acid	-4.60	-4.74	-6.06	-4.82	-5.05	-7.74
Protocatechuic acid	-4.41	-4.56	-6.02	-4.70	-4.92	-6.55
Gentisic acid	-4.38	-3.76	-5.83	-4.90	-5.73	-6.13
Pyrocatechol	-4.80	-4.86	-3.76	-4.06	-5.45	-4.48
Galanthamine	-4.79	-4.87	-3.84	-4.06	-5.35	-4.54
p-Hydroxybenzoic acid	-4.12	-4.26	-6.07	-4.79	-5.77	-6.15
Dihydroxybenzaldehyde	-5.77	-5.45	-4.70	-4.70	-6.60	-5.76
Catechin hydrate	-8.67	-7.71	-6.29	-6.86	-6.97	-9.21
Vanillic acid	-4.54	-4.46	-6.14	-4.88	-4.75	-7.17
Caffeic acid	-4.94	-5.10	-6.67	-5.57	-5.63	-8.22
Syringic acid	-4.85	-4.82	-6.15	-4.69	-5.36	-7.99
Vanillin	-5.87	-5.06	-4.56	-4.35	-6.03	-5.72
Epicatechin	-8.61	-8.41	-6.36	-6.93	-7.06	-9.83
p-Coumaric acid	-4.96	-4.33	-6.65	-5.44	-5.36	-8.99
Ferulic acid	-5.14	-4.37	-6.49	-5.41	-5.47	-8.01

Compound	Fungal target enzymes					
	Candida Albicans		*Blastomyces dermatitidis*		*Ganoderma microsporum*	
	(PDB: 3N9K)	(PDB: 3O6A)	(PDB: 6C85)	(PDB: 6C8W)	(PDB: 3KCW)	(PDB: 4ZIC)
	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)	Score (Kcal/mol)
Catechin gallate	-11.46	-10.43	-8.55	-7.49	-8.89	-12.48
Rutin	-9.90	-9.44	-6.78	-7.88	-10.35	-14.34
trans-2-hydroxycinnamic	-4.80	-5.02	-6.57	-5.25	-5.14	-6.22
Myricetin	-8.59	-9.44	-6.87	-5.43	-7.39	-10.61
Resveratrol	-7.96	-7.81	-5.98	-6.19	-6.54	-8.98
Trans-Cinnamic acid	-4.07	-4.08	-6.30	-5.01	-4.70	-6.72
Luteolin	-8.39	-8.15	-6.97	-7.04	-6.91	-10.28
Quercetin	-8.73	-9.05	-6.91	-6.68	-7.40	-10.95
Naringenin	-8.05	-8.26	-6.04	-7.05	-7.40	-9.55
Genistein	-8.13	-7.92	-6.63	-6.80	-5.95	-8.56
Apigenin	-8.05	-8.08	-6.76	-6.21	-6.55	-9.57
Kaempferol	-8.07	-8.67	-6.44	-6.40	-7.01	-10.30
Hesperetin	-8.22	-8.01	-6.86	-6.15	-6.46	-9.16
Chlorogenic acid	-7.10	-6.28	-7.86	-6.22	-7.54	-11.58
Gallic acid	-4.44	-4.38	-5.63	-4.85	-5.84	-6.90
Chrysin	-8.06	-8.19	-6.86	-6.41	-6.07	-9.29
Rhamnetin	-8.16	-8.17	-6.83	-6.86	-7.19	-11.02
Ketoconazole	-8.99	-9.30	-7.32	-9.51	-8.79	-12.58
Griseofulvin	-8.01	-7.88	-6.34	-7.06	-6.79	-8.14

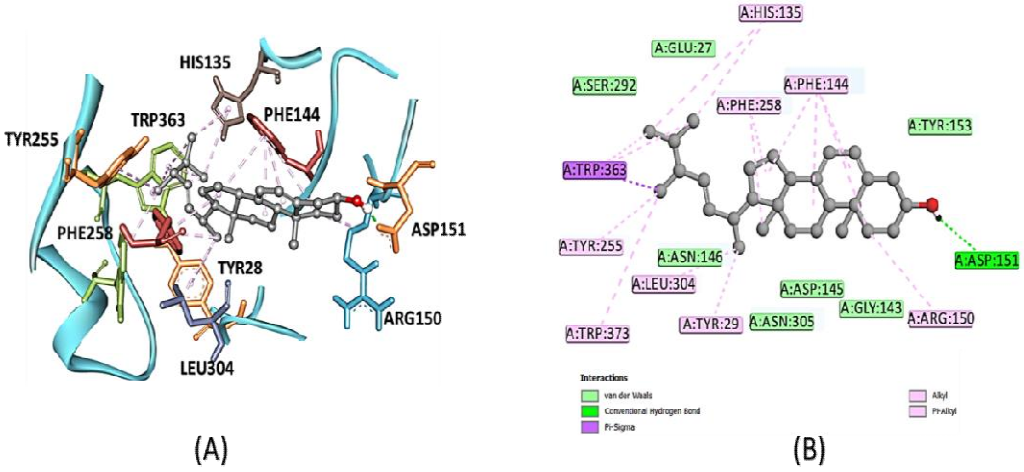
4. DISCUSSION

The docking scores showed there are some phytoconstituents having high binding affinities for the fungal proteins disproportionately. The binding energy of *Candida Albicans* (3N9K, 3O6A) with ergosterol were -11.75 and -11.37 kcal/mol, and it was found for catechin gallate equal to -11.46 and -10.43 kcal/mol)respectively, and for rutin it found to be -9.90 and -9.44 kcal/mol and it showed more negative binding energy than the controls (Table 2). Hydrophobic interaction represents the main type of interactions between the hydrophobic backbone of ergosterol and the amino acids inside the active site of *Candida Albicans* protein (3NK9.PDB). In addition, the hydroxyl group of ergosterol formed a hydrogen bond with ASP151. On the contrary, the structures of catechin gallate and rutin are rich with hydroxyl groups, thus hydrogen bonds are the main type of interaction for both compounds. Catechin gallate was capable to form 10 hydrogen bonds with GLU192, TYR255, HIS135, GLU27, ASN191, SER292, ASP145, ARG309, and GLY143 (Fig. 1C and D). Similar to that, rutin was also capable to form 10 hydrogen bonds with AGR312, PHE258, TYR255, GLN192, GLY143, and AGR309 (Fig. 1E and F). In addition to hydrogen bonds, hydrophobic interactions with the lipophilic side chain of the amino acids inside the active site of *Candida Albicans* protein (3N9K.PDB) also exist.

A similar mode of interactions was represented for ergosterol, catechin gallate, and rutin in complex with Candida Albicans (PDB: 3O6A), where the interactions mode displayed that the hydrophobic interaction is the predominant type of interaction for ergosterol (Fig. 2A and B), and hydrogen bonding is the main type of interactions in case catechin gallate (Fig. 2C and D), and rutin (Fig. 2E and F). On the other hand, the molecular docking study of the truffle's components against Blastomyces dermatitidis (PDB: 6C85) showed that ergosterol and chlorogenic acid ranked the highest (-8.54 and 7.86 kcal/mol respectively) while in the case of the other crystal structure of the protein (PDB: 6C8W), ergosterol score the highest binding energy (-9.42 kcal/mol) which is relatively comparable to that of ketoconazole (-9.51 kcal/mol) but higher than griseofulvin (-7.06 kcal/mol).

Fig. 3 represents the binding mode of ergosterol inside the active site of both crystal structures of aspartate semialdehyde dehydrogenase from Blastomyces *dermatitidis* (PDB: 6C85 and 6C8W). Ergosterol backbone is highly lipophilic, thus hydrophobic interactions predominantly exist. In addition, the hydroxyl group of ergosterol formed a hydrogen bond with ASN 109 (Fig. 3A and B, PDB: 6C85) and SER152 (Fig. 3C and D, PDB: 6C8W).

The potential activity of the truffles phytoconstituents against *Ganoderma microsporum* fungal protein revealed that the ergosterol, catechin gallate, and rutin showed the lowest binding energy scores ranging from -10.35 to -8.89 kcal/mol against the protein structure (PDB: 3KCW). And the scores showing the free binding energy against (PDB:4ZIC) were -14.34 to-12.48 kcal/mol, with rutin ranked the highest affinity to binding to the active site



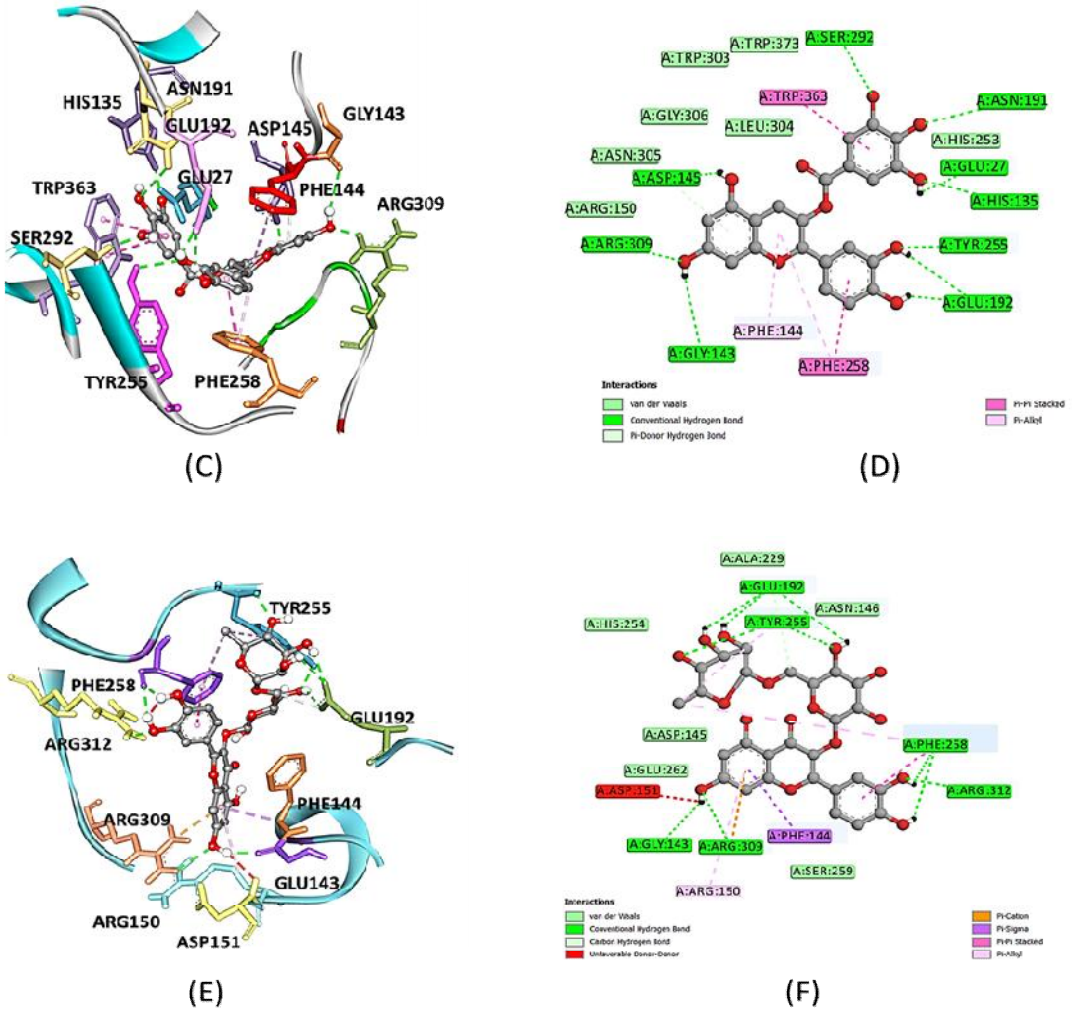


Fig. 1. Binding Interaction mode of functional groups of desert truffles molecules ergosterol (A and B), catechin gallate (C and D), and rutin (E and F) with the *Candida Albicans* receptor (3N9K.PDB) in 3D and 2D model, respectively. The green dash line represents the hydrogen bonding interaction, pink and purple hydrophobic interactions, and the red line represents the steric interaction

compared to the controls. The docked structures of ergosterol, catechin gallate, and rutin with *microsporum* proteins (PDB: 3KCW and PDB:4ZIC) are represented in Figs. 4 and 5, respectively. In Fig. 4, ergosterol formed a hydrophobic interaction with the side chain of TYR21, PHE19, VAL107, VAL96, PHE105, TYR48, LEU43 amino acids. Besides, the hydroxyl group of ergosterol formed a hydrogen bond with THR22 (Fig. 4A and B). Catechin gallate formed a hydrophobic interaction with the hydrophobic side chains of the amino acids inside the active site of the protein such as PHE105, TYR21, TYR48, LEU43, and VAL 96, while it also formed two hydrogen bonds with VAL107 and ILE106 amino acids (Fig. 4C and D). Rutin formed 5 hydrogen bonds with ILU106, VAL107, TYR21, and TYR48 in addition to hydrophobic interactions with the hydrophobic side chains of amino acids inside the active site such as PHE 105, TYR48, and TYR21 (Fig. 4E and F).

Ergosterol (Fig. 5A and B) formed hydrophobic interactions with VAL192, ALA187, CYC154, HIS 251, ILU205, and PRO206 while it formed a

hydrogen bond with ASP196. Catechin gallate formed a hydrophobic interaction with the ALA187 and PRO206 hydrophobic side chains while it also formed four hydrogen bonds with LYS111, LYS211, ASN153, and GLU208 amino acids (Fig. 5C and D). Rutin, which showed the highest binding affinity (-14.34 kcal/mol), formed 7 hydrogen bonds with LYS211, PRO206, ARG244, GLY186, ALA17, AND 189 in addition to hydrophobic interactions with the hydrophobic side chains of CYS154, and PRO206 amino acids (Fig. 5E and F). Rutin also formed a favorable anion-π interaction with GLU208 and a favorable electrostatic cation-π interaction with LYS211.

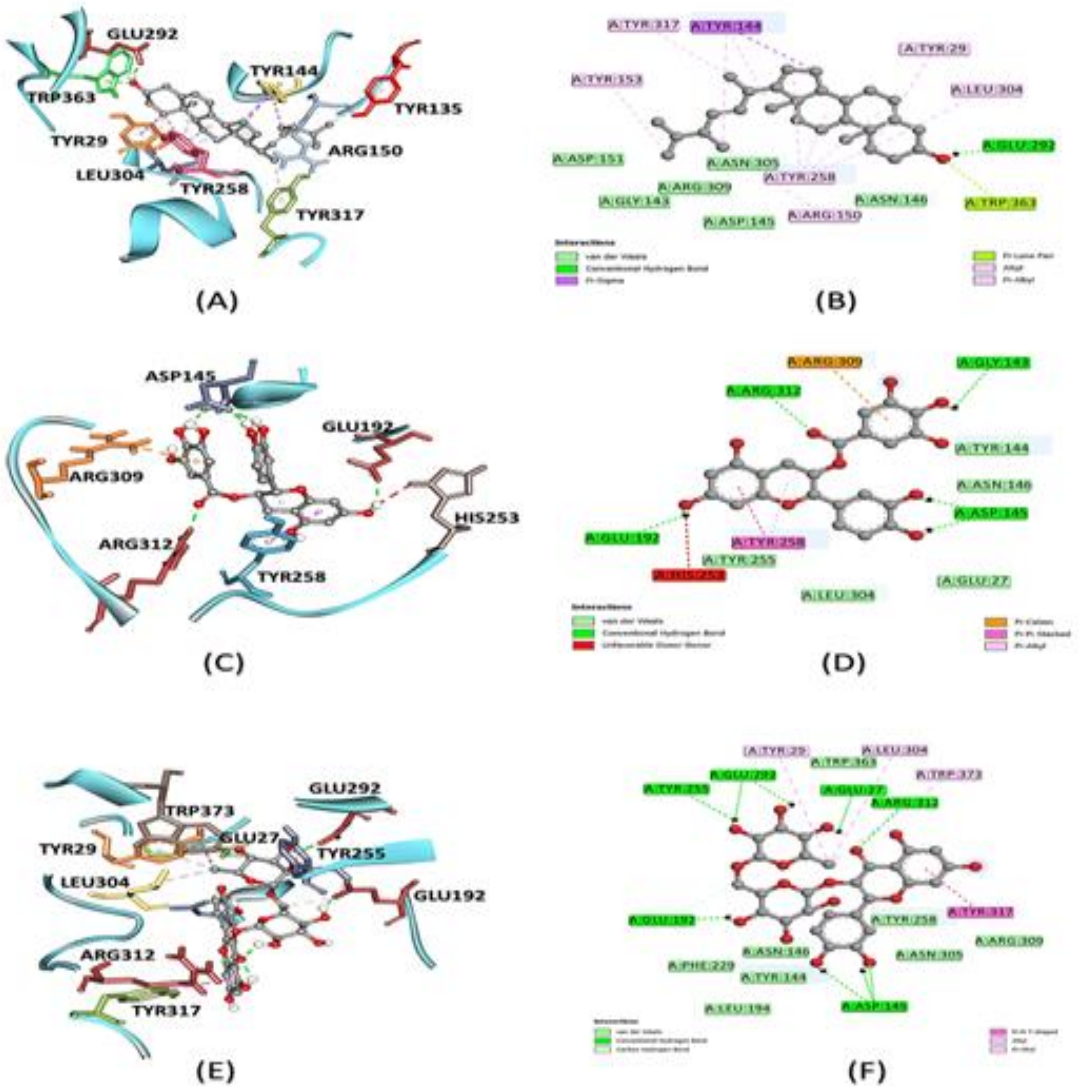


Fig. 2. Binding Interaction mode of functional groups of desert truffles molecules ergosterol (A and B), catechin gallate (C and D), and rutin (E and F) with the *Candida Albicans* receptor (PDB: 3O6A) in 3D and 2D model, respectively. The green dash line represents the hydrogen bonding interaction, pink and purple hydrophobic interactions, and the red line represents the steric interaction

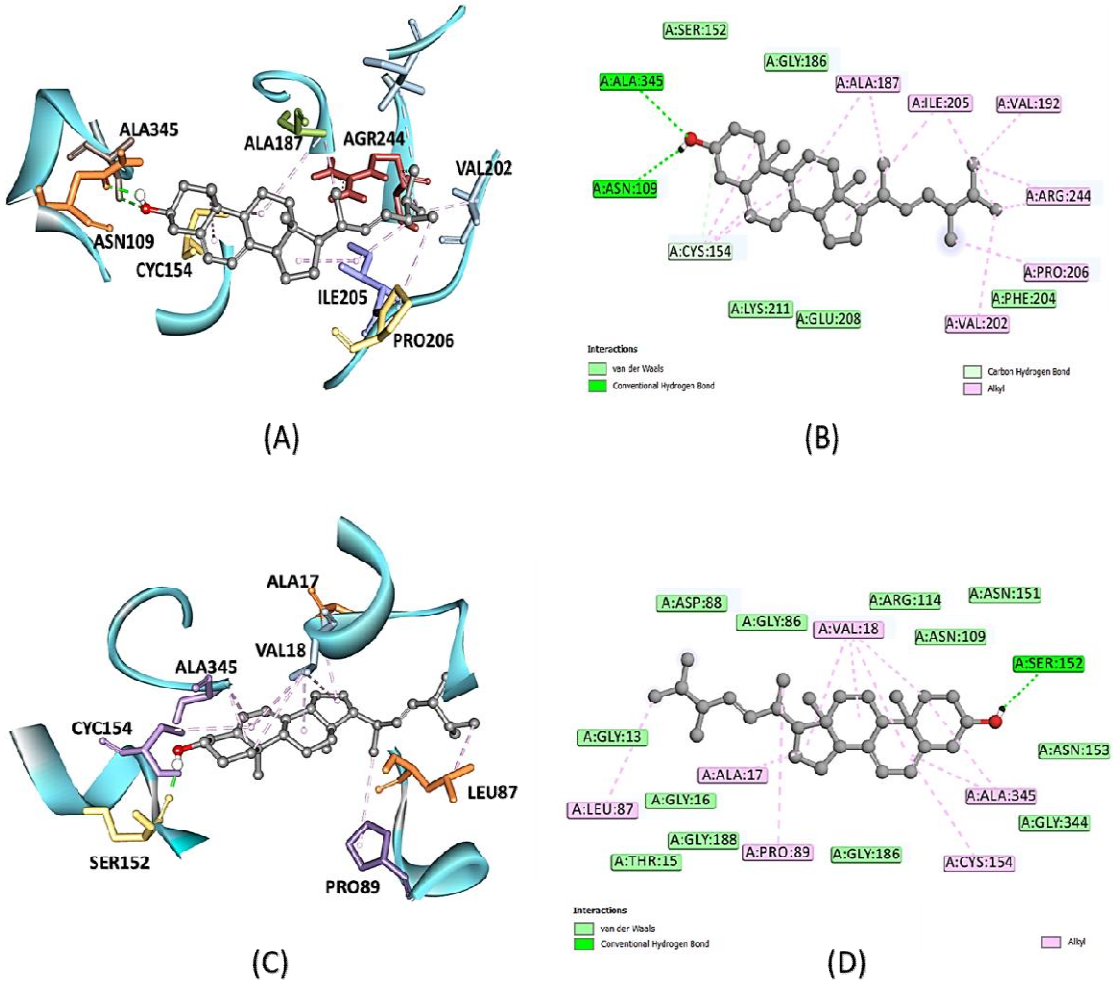
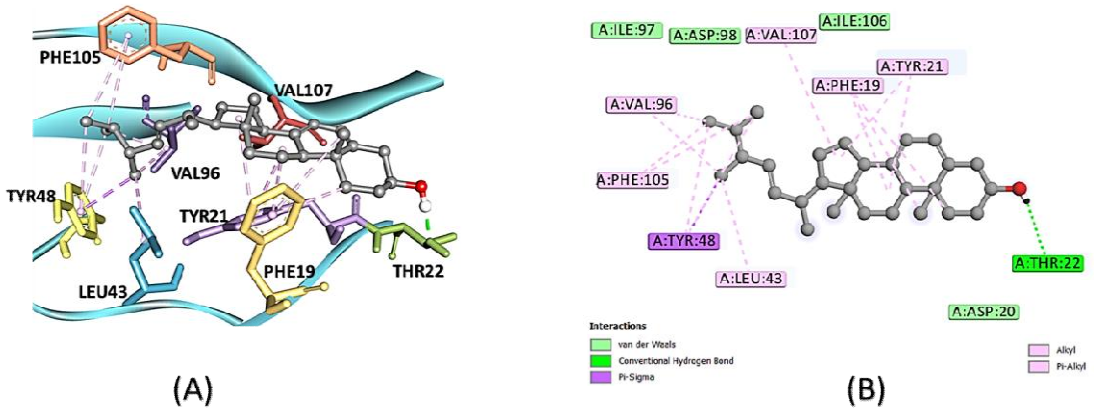


Fig. 3. Binding Ergosterol Interaction Mode with Blastomyces *dermatitidis* PDB: 6C85 (A and B) and (C and D) with PDB: 6C8W The green dash line represents hydrogen bonding interactions, pink and purple are hydrophobic interactions



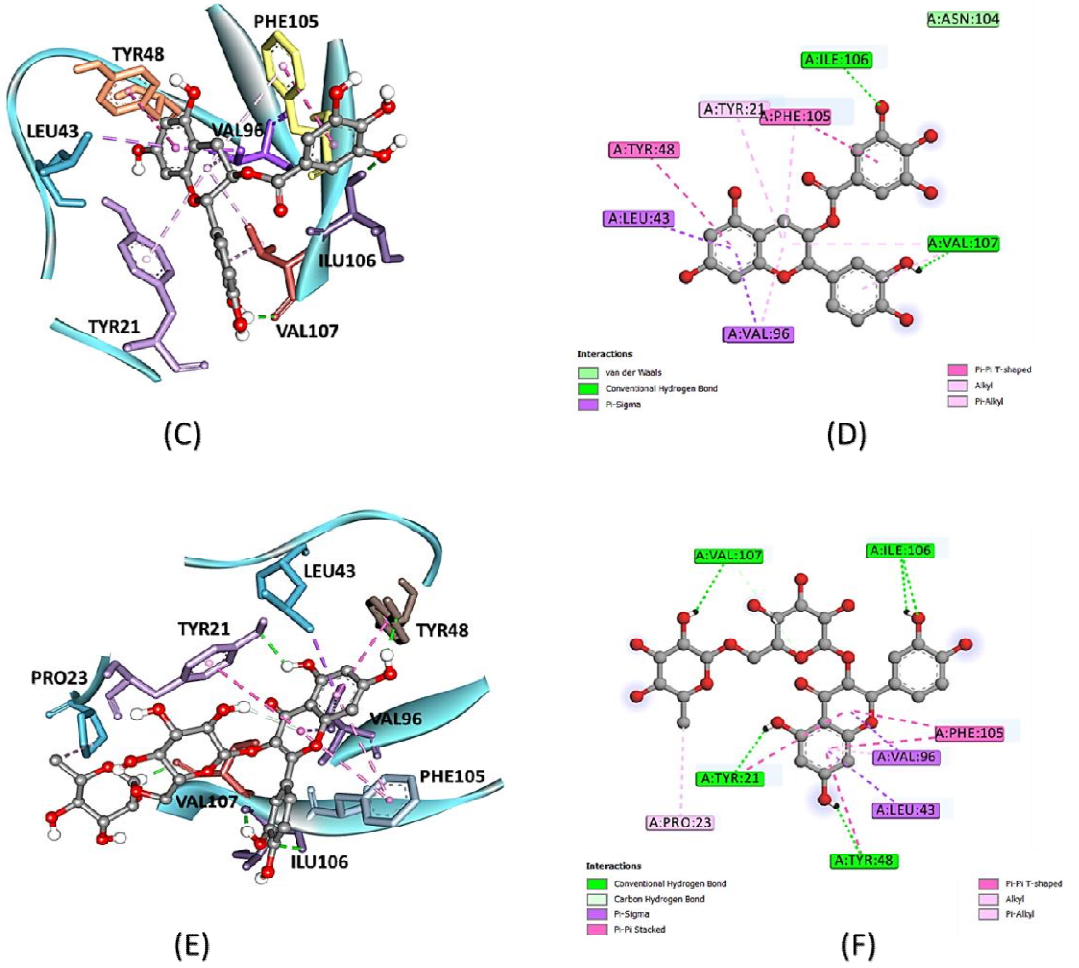
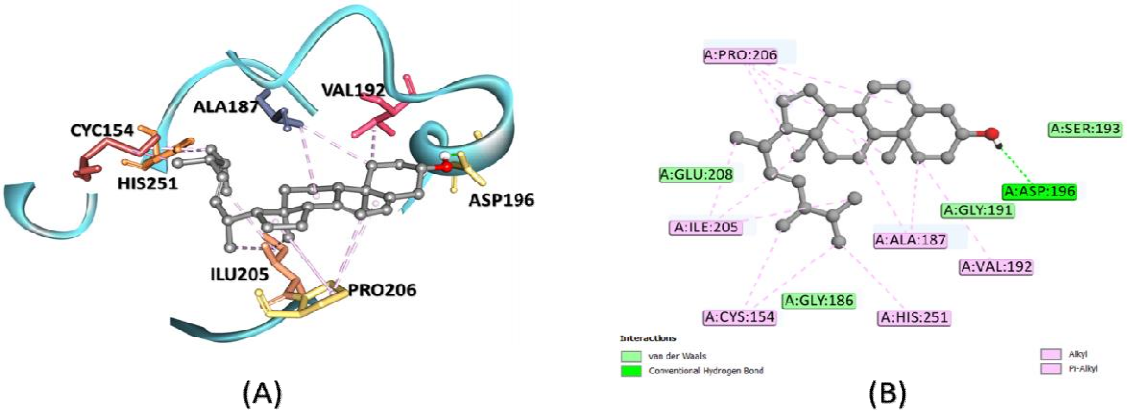


Fig. 4. Binding Interaction mode of desert truffles molecules ergosterol (A and B), catechin gallate (C and D), and rutin (E and F) with the *Ganoderma microsporum* fungal protein (PDB: 3KCW) in 3D and 2D model, respectively. The green dash line represents the hydrogen bonding interaction, pink and purple hydrophobic interactions, and the red line represents the steric interaction



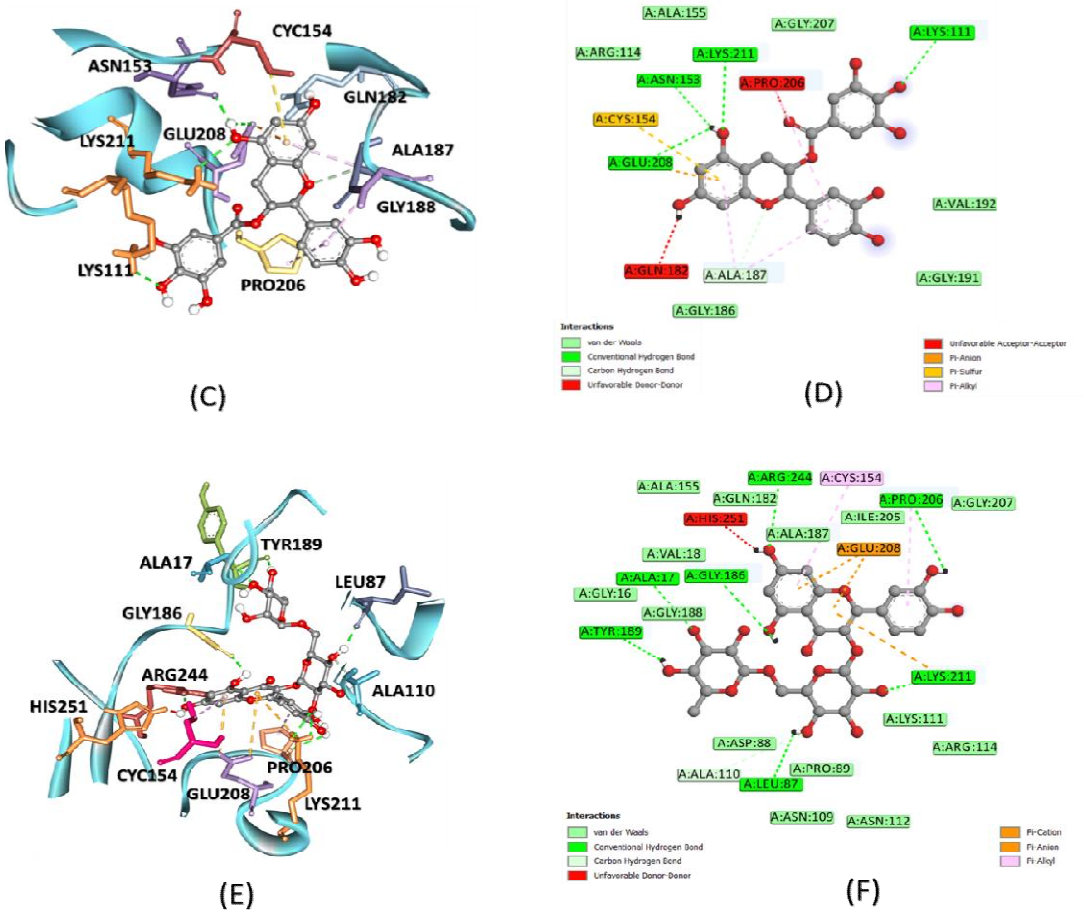


Fig. 5. Binding Interaction mode of desert truffles molecules ergosterol (A and B), catechin gallate (C and D), and rutin (E and F) with the *Ganoderma Microsporum* fungal protein (PDB: 4ZIC) in 3D and 2D model, respectively. The green dash line represents the hydrogen bonding interaction, pink and purple hydrophobic interactions

5. CONCLUSION

In this study, *in silico* molecular docking technique was employed to explore the potential antifungal activity of 44 components from desert truffles. The applied approach and comparing with ketoconazole and griseofulvin showed that ergosterol, catechin gallate, and rutin act as potential antifungal agents. Regarding *Candida Albicans*, ergosterol showed the highest binding energy followed by catechin gallate then rutin. Relatively similar behavior for the same compounds was noticed against Blastomyces *dermatitidis* (PDB: 6C85 and 6C8W), *Ganoderma microsporum* fungal protein where they showed the most negative binding energy among the other truffle's components. Ergosterol showed the highest binding energy against Blastomyces *dermatitidis* while on the other hand, rutin scored the lowest against *Ganoderma microsporum*. *In vitro* analysis of the potential anti-fungal of the desert truffles components is still to be conducted in the future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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