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Research Article

In silico screening of phytochemicals against Ypd1 protein of a destructive storage fungi, *Aspergillus*

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ABSTRACT: One of the most common pests in stored grain is *Aspergillus*. This group of fungi produces a carcinogenic toxin, Aflatoxin during their growth and development. Contamination of *Aspergillus* in food grains during storage leads to food insecurity. In the present-day scenario, using plant-based derivatives in controlling *Aspergillus* is one of the efficient and eco-friendly ways. Hence an *in silico* study was carried out to know the effective phytochemicals present in *Citrus, Carum carvi, Coriander sativum, Aloysia citriodora, Mentha citrate,* Spent hops*, Nardostachys jatamansi, Feoniculum vulgare, Zingiber officinale, Lantana camara, Chamaecyparis obtusa, Ocimum kilimandscharium, Tagetes filifolia* against *Aspergillus*. Results revealed that the photochemicals viz. Eugenol, zingiberene, carvone, citronellal, limonene, coumaran, linalool, linalyl acetate, esdragol, menthol, E-anethole, camphor, bornyl acetate, xanthohumol and aristolone present in the selected plants can inhibit the normal functioning of Ypd1 protein of *Aspergillus* by blocking its active site. Thus, the present study makes a base for future researchers to find the most effective phytochemicals in controlling *Aspergillus* following *in vivo* method.

KEYWORDS: Aflatoxin, botanical, molecular docking, phytochemical, storage fungi

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INTRODUCTION

Post-harvest loss is a hurdle in doubling the income of farmers. During the process, a huge amount of food grain is lost due to improper management. Globally, postharvest losses account for 24% of the total food produced and it varies from about 9% in developed countries to more than 20% in developing countries (Phillips & Throne, 2010). Therefore, food safety is an essential factor regarding food demand for the growing population across the world. Storage of food grain is an important post-harvest process. According to the Food Corporation of India, storage is the major cause of post-harvest losses for all kinds of food which is estimated around 15% of the total food production (Trade Promotion Council of India, 2020). Kumar and Kalita (2017) reported that 50-60% of cereal grains can be lost during the storage stage due only to the lack of technical inefficiency. Contamination of pests during storage of food grain is one of the major factors of post-harvest loss. Stored grains are severely damaged by insects. Contamination of insect pests causes damage to stored grains resulting in both qualitative and quantitative losses. The majority of stored grain pests belong to two orders, i.e., Coleoptera and Lepidoptera accounting for almost 60 and 10% respectively (Khare, 1994). Worldwide stored grain pests pose a serious threat to dried, stored, durable and perishable agricultural products and nonfood derivatives of agricultural products. Almost 8–10% (13 million tons) of grains were lost due to insects and 100 million tons due to improper storage. Pests such as various insects, pathogens, and mites possess serious threats and cause severe damage to grains by producing certain enterotoxins and mycotoxins (Magan & Aldred, 2007). In developing countries, the greatest losses during storage of cereals, pulses and other durable commodities are caused by storage fungi. Storage fungi mostly invade the grains during the period of storage due to improper storage and unhygienic conditions, even the smallest number of inoculums can spoil the entire stored product. The food seeds such as wheat, and rice are stored for varying lengths of time for various purposes and it was estimated that approximately 10–20% of the stored seeds become deteriorated by fungi (Kumar *et al.,* 2023). Storage fungi can cause a decrease in germination capability, loss in weight, discolouration of kernels, heating and mustiness, chemical and nutritional changes and mycotoxin contamination (Sauer *et al.,* 1998; Bulaong & Dharmaputra, 2002). Microorganisms invade the seed, on the crop during crop growth in the field, during harvesting and post-harvest handling or storage and distribution (Barth *et al.*, 2009). Mycotoxins are a common problem for stored grains, fruits and vegetables (Bartholomew *et al.,* 2021). Aflatoxin, a major mycotoxin, is significant due to its deleterious effects on human beings, poultry and livestock (Abbas 2005; Chaytor *et al.,* 2011). It is a potent carcinogenic, mutagenic, and immunosuppressive agent (Zain, 2011), produced as secondary metabolites by the fungi, *Aspergillus flavus*, *A. parasiticus* and *A. nomius* on a variety of food commodities (Essono *et al.,* 2009). Infection of *A. flavus* and subsequent aflatoxin contamination in groundnut can occur at pre-harvest, harvest, and post-harvest storage and processing (Harish *et al.,* 2014). Due to the production of mycotoxins storage, the seeds become unfit for human consumption and there is a reduction in their market value (Muller, 1991).

Strategies for mitigating post-harvest losses could be a sustainable way to improve food security. Different approaches have already been made to control and prevent mycotoxin in food and feed (Makhuvele *et al.,* 2020). Application of chemicals for its control may be effective but it leads to issues like resistance, resurgence and poisoning of food grain. Secondary metabolites derived from the plants may be considered a better alternative to conventional chemical application. The phytochemicals are specific to target species and have fewer negative effects on other organisms. Moreover, the active ingredient of the plants degrades rapidly and hence resistance to these compounds is not developed by the target organisms (El-Wakeil, 2013). Relevant works on searching for effective phytochemicals in controlling various insect pests have been done by different workers in due course of time. The list of effective phytochemicals in controlling insect pests has already been well documented and their effectiveness was confirmed. The work on the effectiveness of these phytochemicals in controlling *Aspergillus* is not adequate. Hence, the present *in silico* study was carried out to know whether the phytochemicals that are effective in controlling insect pests can also control the *Aspergillus* or not.

MATERIALS AND METHODS

Collection of secondary data: Secondary data established that phytochemicals derived from the plants viz. *Citrus, Carum carvi, Coriander sativum, Aloysia citriodora, Mentha citrate,* Spent hops, *Nardostachys jatamansi, Feoniculum vulgare, Zingiber officinale, Lantana camara, Chamaecyparis obtusa, Ocimum kilimandscharium, Tagetes filifolia* have the potentiality to control different insect pest of stored grain (Singh *et al.,* 2021) and the data on effectiveness of these phytochemicals in controlling *Aspergillus* is inadequate.

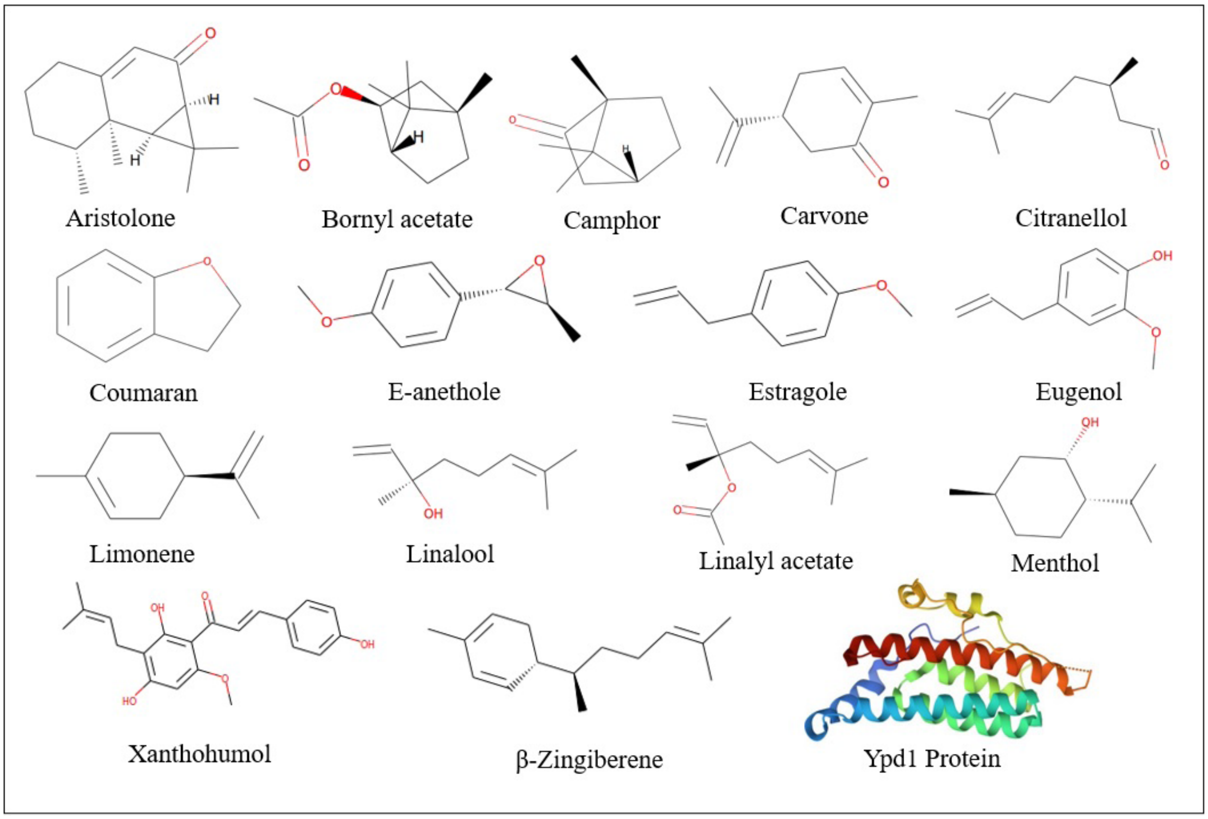

Figure 1. The structures of the ligands (phytochemicals) present in selected plant extracts were downloaded from the Zinc database (https://zinc.docking.org). The ligands taken for the present study were selected from previously published literature.

In silico analysis to know the efficacy of selected plant extract

At the beginning of the present study *in silico* techniques were used to know the effectiveness of selected plant extract in controlling the target fungi. The analysis was performed using important enzymes of the target fungi as receptors. Ypd1 protein was selected for the *in silico* study. The Ypd1 protein is important for the physiology of selected fungi as it contributes to the ability of the pathogen to adapt to various stressed conditions (Schruefer, 2021). The phytochemicals (ligands) from the *Punica granatum* were selected to analyze their inhibition ability to the functioning of the selected protein. The possible positive result i.e., positive binding affinity of selected phytochemicals with selected enzymes can give us insight regarding the use of those phytochemicals for controlling the target fungi.

Receptor and ligand preparation

The molecular structures of the receptor (enzymes) Ypd1 (1C03) (Figure 1) have been downloaded from the PDB database (https://www.rcsb.org/).

Molecular docking

For docking, Molegro Virtual Docker (MVD 2010.4.0.0) for Windows was used to know the possible molecular interactions between the receptors (enzymes) and the ligand molecules (phytochemicals). Rerank scores were taken into account for analyzing the receptor-ligand interactions. It uses energy parameters such as E-Inter total, E-Inter (protein-ligand), Steric, Van der Waal's, H-Bond energy, E-Intra (tors, ligand atoms) etc.

Receptor-ligand interactions visualization

For visualization, BIOVIA Discovery Studio Visualizer 2021 was used. Dassault Systems BIOVIA 2021 developed this program used for viewing, sharing and analyzing protein and small molecules.

Statistical analysis: SPSS v 15 (SPSS Inc, Chicago, IL, USA) software was used for statistical analysis.

RESULTS

The *in silico* result showed that eugenol exhibited highest rerank score (-65.29±6.50) followed by beta zingiberene (-56.23±7.40), carvone (-53.47±13.54), citronellal (-52.22±5.71), limonene (-51.89±6.85), coumaran (-51.75±6.45), linalool (-49.95±10.11), linalyl acetate (-49.72±8.59), esdragol (-49.27±10.53), menthol (-47.47±11.35), E-anethole (-45.23±13.32), camphor (-45.16±9.05), bornyl acetate (-44.34±14.09), xanthohumol (-32.59±25.10) and aristolone (-31.94±11.39) (Table1). The graphical representation of the rerank score is shown in Figure 2. These values indicate that the selected phytochemicals used in the present study have the potential to inhibit normal enzyme activity.

The 3D and 2D interactions of selected phytochemicals with the protein show different types of interactions like Van der Waals, H-Bond, C-H Bond, Non-covalent bonds like Pi-Alkyl bond, Pi-pi T shaped, Pi- Sulphur bond, etc. The top three 3D and 2D representations of interaction between selected phytochemicals with the active site of Ypd1 protein are shown in Figure 3.

Table 1. Table showing rerank scores of selected ligands against Ypd1 Protein

Name of ligand	ZINC ID	Mean Rerank Score
Eugenol	ZINC1411	-65.29±6.50
Carvone	ZINC14588455	-53.47±13.54
Linalool	ZINC1529820	-49.95±10.11
Citronellal	ZINC1531600	-52.22±5.71
Linalyl acetate	ZINC2041035	-49.72±8.59
Menthol	ZINC4228277	-47.47±11.35
Xanthohumol	ZINC5158937	-32.59±25.10
Aristolone	ZINC6030836	-31.94±11.39
E-anethole	ZINC6066878	-45.23±13.32
Beta-Zingiberene	ZINC62233813	-56.23±7.40
Coumaran	ZINC6661321	-51.75±6.45
Bornyl Acetate	ZINC84758359	-44.34±14.09
Limonene	ZINC967513	-51.89±6.85
Camphor	ZINC967520	-45.16±9.05
Esdragol	ZINC967635	-49.27±10.53

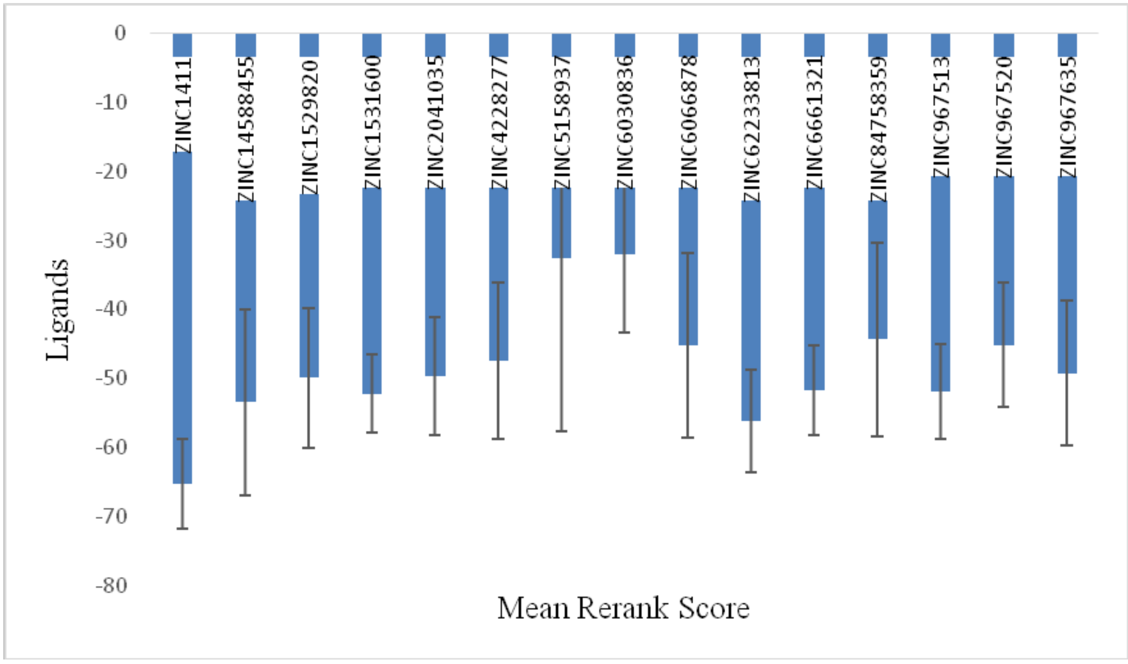

Figure 2. Graph showing rerank score of different ligands with Ypd1 protein.

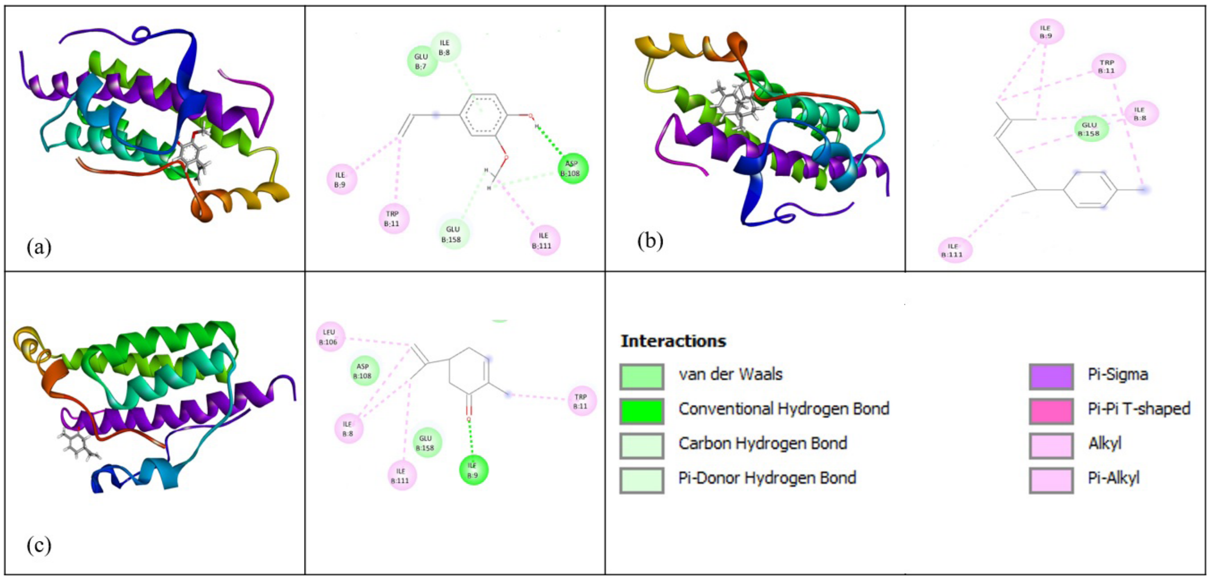

Figure 3. 3D and 2D representations of **(a).** Interaction of eugenol with the active site of Ypd1 protein, **(b).** Interaction of beta zingiberene with the active site of Ypd1 protein, **(c).** Interaction of carvone with the active site of Ypd1 protein.

The physicochemical properties of selected ligands are depicted in Table 2. The pharmacokinetics of selected ligands was studied and has been observed that the selected ligands have the potential to be considered effective drug properties which gives an insight into the efficacy of selected ligands. The results show that out of fifteen ligands taken in the present study, thirteen ligands namely Eugenol, Carvone, Linalool, Citronellal, Linalyl acetate, Menthol, Xanthohumol, Aristone, E-anethole, Coumaran, Bornyl acetate, Limonene and Camphor have the property of GI absorption. Eugenol, Carvone, Linalool, Citronellal, Linalyl acetate, Menthol, Xanthohumol, Aristone, E-anethole, Beta zingiberene, Coumaran, Bornyl acetate and Limonene can cross the Blood-brain barrier. Menthol, Xanthohumol, Aristone, E_ anethole and camphor can act as CYP1A2 inhibitors. Eugenol and Esdragol can act as CYP2C19 inhibitors. Eugenol, beta zingiberene, camphor, and esdragol can act as CYP2C9 inhibitors. Camphor can act as CYP3A4 inhibitor.

DISCUSSION

The literature revealed that the presence of mycotoxin in the stored grains is a serious concern for food safety. These

Table 2. Physiochemical properties of selected ligands

Sl. No.	Zinc ID	Name of Ligand	Physicochemical Parameters								
			Formula	Molecular Weight	No. of Heavy atoms	No. of Aromatic heavy atoms	Fraction Csp3	No. of Rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	Molecular refractivity	TPSA
1	ZINC1411	Eugenol	C15H22O	218.33	16	0	0.8	0	1	0	67.08	17.07
2	ZINC14588455	Carvone	C12H20O2	196.29	14	0	0.92	2	2	0	56.33	26.3
3	ZINC1529820	Linalool	C10H16O	152.23	11	0	0.9	0	1	0	45.64	17.07
4	ZINC1531600	Citronellal	C10H14O	150.22	11	0	0.5	1	1	0	47.32	17.07
5	ZINC2041035	Linalyl acetate	C10H18O	154.25	11	0	0.7	5	1	0	49.91	17.07
6	ZINC4228277	Menthol	C8H8O	120.15	9	6	0.25	0	1	0	35.79	9.23
7	ZINC5158937	Xanthohumol	C10H12O2	164.2	12	6	0.4	2	2	0	46.48	21.76
8	ZINC6030836	Aristolone	C10H12O	148.2	11	6	0.2	3	1	0	47.04	9.23
9	ZINC6066878	E-anethole	C10H12O2	164.2	12	6	0.2	3	2	1	49.06	29.46
10	ZINC62233813	Beta-Zingiberene	C10H16	136.23	10	0	0.6	1	0	0	47.12	0
11	ZINC6661321	Coumaran	C10H18O	154.25	11	0	0.6	4	1	1	50.44	20.23
12	ZINC84758359	Bornyl Acetate	C12H20O2	196.29	14	0	0.58	6	2	0	60.17	26.3
13	ZINC967513	Limonene	C10H20O	156.27	11	0	1	1	1	1	49.23	20.23
14	ZINC967520	Camphor	C21H22O5	354.4	26	12	0.19	6	5	3	102.53	86.99
15	ZINC967635	Esdragol	C15H24	204.35	15	0	0.6	4	0	0	70.68	0

Table 3. Pharmacokinetics of selected ligands

Sl. No.	Zinc ID	Name of Ligand	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)
1	Eugenol	Eugenol	High	Yes	No	No	Yes	Yes	No	No	-5.08
2	Carvone	Carvone	High	Yes	No	No	No	No	No	No	-5.31
3	Linalool	Linalool	High	Yes	No	No	No	No	No	No	-5.67
4	Citronellal	Citronellal	High	Yes	No	No	No	No	No	No	-5.29
5	Linalyl acetate	Linalyl acetate	High	Yes	No	No	No	No	No	No	-4.52
6	Menthol	Menthol	High	Yes	No	Yes	No	No	No	No	-5.51
7	Xanthohumol	Xanthohumol	High	Yes	No	Yes	No	No	No	No	-6.03
8	Aristolone	Aristolone	High	Yes	No	Yes	No	No	No	No	-4.81
9	E-anethole	E-anethole	High	Yes	No	Yes	No	No	No	No	-5.69
10	Beta-Zingiberene	Beta-Zingiberene	Low	Yes	No	No	No	Yes	No	No	-3.89
11	Coumaran	Coumaran	High	Yes	No	No	No	No	No	No	-5.13
12	Bornyl Acetate	Bornyl Acetate	High	Yes	No	No	No	No	No	No	-4.71
13	Limonene	Limonene	High	Yes	No	No	No	No	No	No	-4.84
14	Camphor	Camphor	High	No	No	Yes	No	Yes	No	Yes	-4.86
15	Esdragol	Esdragol	Low	No	No	No	Yes	Yes	No	No	-3.88

toxins are mainly produced by fungi during their growth and reproduction. Among the mycotoxins, aflatoxin is one of the carcinogenic toxins which cause health issues. Control of these toxins is only possible by controlling the appearance of fungi in storage. Different conventional chemical control measures have already been implemented which leads to food poisoning. Studies on the replacement of chemical pesticides by phytochemicals for controlling different insect pests have been done by different authors in due course of time. Their study reveals that different phytochemicals have the potential to control different insect pests to varying degrees. The study on the effectiveness of phytochemicals in controlling *Aspergillus* is inadequate. Present *in silico* study reveals the potentiality of different phytochemicals in controlling *Aspergillus* during storage. Eugenol, a phytochemical present in *Citrus* shows highest rerank score followed by beta zingiberene (*Zingiber officinale*), carvone (*Mentha citrata*), citronellal (*Aloysia citriodora*), limonene (*Citrus*), coumaran (*Lantana camara*), linalool (*Mentha citrata*), linalyl acetate (*Mentha citrata*), esdragol (*Feoniculum vulgare*), menthol (*Mentha citrata*), E-anethole (*Carum carvi*), camphor (*Rosmarinus officinalis*), bornyl acetate (*Chamaecyparis obtusa*), xanthohumol (Spent hops) and aristolone (*Nardostachys jatamansi*) which ranges from -65.29 to -31.94. In a study, Aamir *et al.* (2018) reported that Oxathiapiprolin and Famoxadone have a good binding affinity against short-chain dehydrogenases and they established them as better fungicides in their *in silico* analysis. In another study, Bouqellah (2023) found that the nanoparticles were able to bind to sterol 14-alpha demethylase responsible for inhibiting ergosterol biosynthesis and can control fungi. Camptothecin and GKK1032A2 showed excellent binding energy with the target protein of *Magnaporthe oryzae* (Khan *et al.*, 2023).

CONCLUSION

Aspergillus is one of the major devastating genera among the fungi during the storage. Controlling this fungus organically is the best and most eco-friendly way for sustainable agriculture. Our *in silico* study confirmed that the eugenol, carvone, linalool, citronellal, linalyl acetate, menthol, xanthohumol, aristolone, e-anethole, beta_ zingiberene, coumaran, bornyl acetate, limonene, camphor, esdragol present in different plants have the potentiality to inhibit the normal functioning of Ypd1, an important protein of *Aspergillus* sp. This study paves the way for future researchers to validate the *in silico* study in wet lab analysis and can establish the most effective phytochemicals in controlling *Aspergillus*.

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