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Research Article

ANTIMICROBIAL ACTIVITY OF DIETHYL PHTHALATE: AN INSILICO APPROACH

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ABSTRACT

Background: Reactive oxygen species (ROS)are produced by host phagocytes and can attack a diverse range of targets to exert antimicrobial activity, against a broad range of pathogens. Four major ROS are recognized comprising superoxide, hydrogen peroxide, hydroxyl radical (•OH), and singlet oxygen. Antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and glutathione reductase (GSH) plays a crucial role by protecting the organism from endogenous ROS. This work is designed to find out the dominant secondary metabolites derived from the fungus *Aspergillus* sp. isolated from *Lannea coramendalica* and to elucidate antimicrobial activity through interaction with specifically intracellular targets.

Materials and Methods: *Aspergillus* sp. was isolated and inoculated in a production media to extract secondary metabolite by using ethyl alcohol as solvent. The filtrate was analysed for secondary metabolites by using gas chromatography/mass spectrometry (GC-MS) analysis. The compound diethyl phthalate identified by GC-MS analysis was screened against the target protein. Retrieved from the protein data bank and docking analysis were studied using Autodock and Lig Plot software.

Results: While comparing the results from the docking experiments and their interactions among the three enzymes SOD has the least binding energy of −6.45 kcal and more stable with the ligand diethyl phthalate after it has been docked. Anti -oxidant function of SOD is inhibited more rather than the other two proteins GPX and GSH reductase. As a result there is an increase in superoxide production.

Conclusions: The increase in superoxide production, exert ROS generated oxidative stress in the cytoplasm of bacterial cells, leading to cell death. Hence diethyl phthalate could be used as a potent antimicrobial agent.

Keywords: Endophytic fungi, Diethyl phthalate, Reactive oxygen species, Antioxidant enzymes.

INTRODUCTION

Endophytic fungi are group of microorganism colonized in inter and intracellular host plants without causing any disease, plays important physiological roles and ecological roles [1]. Endophytes provide a broad variety of bioactive secondary metabolites which possess various biological activities with roles as defensive compounds against competitors/parasites/predators, growth and reproduction facilitators, or as cell signaling compounds [2] So far, studies reported a large number of antimicrobial compounds isolated from endophytes, belonging to several structural classes such as alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids [3]. In general, antimicrobials are thought to kill microbes through interaction with specifically intracellular targets, followed by corruption of particular cellular processes [4]. In recent times, it has been demonstrated that major classes of bactericidal antibiotics, irrespective of their drug-target interactions, induce a common oxidative damage cellular death pathway in bacteria, leading to the production of lethal reactive oxygen species (ROS) [5] via disruption of the tricarboxylic acid cycle and electron transport chain (ETC)[6]. Three major ROS, superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), are generated as by-products of normal aerobic respiration [7,8]. Superoxide, a reactive oxygen by-product, generated by leakage of electrons from the ETC to oxygen, is a precursor to many other forms of ROS, one instance being the enzymatic conversion into H_2O_2 through SOD [9] ROS are vital for cells as molecular and key regulators, but they are destructive for cells in high extent of their production. ROS are produced in organelles of chloroplasts, mitochondria and peroxisomes in different metabolic pathways, and they are exterminated by antioxidant defense mechanisms [10]. The antioxidant defense is primarily constituted by the actions of glutathione peroxidase (GPX), SOD, catalase and ascorbate peroxidase [11] Glutathione (GSH) in reproductive tract secretions with the function of GPX, superoxide dismutase and GSH reductase plays a crucial role by protecting the organism from endogenous ROS.

This work is designed to find out the dominant secondary metabolites derived from the fungus *Aspergillus* sp. isolated from *Lannea coramendalica* and to elucidate the changes induced in the enzyme activity of various antioxidant enzymes like SOD, glutathione S-transferase and GPX using in-silico molecular docking methods.

MATERIALS AND METHODS

Aspergillus sp. was isolated from the leaves of *L. coramendalica* by using potato dextrose agar medium and stored at 4°C. The strains of *Aspergillus* sp. were inoculated in a production media and incubated at 28°C for 12 days, and biomass was then harvested. The fungal biomass was extracted for intracellular metabolites by using ethyl acetate as solvent, and this extraction was repeated three times. The crude extract was filtered through Whatman No 1 filter paper, and the filtrate was dried under vacuum at 40°C. The filtrate was analyzed for secondary metabolites by using gas chromatography/mass spectrometry (GC-MS) analysis. After analysis, the compounds were identified by matching with known compound library.

The target protein SOD, GPX and GSH reductase (PDB ID: 2NYB, 2V1M, 3GRS respectively) were retrieved from the protein data bank (PDB)(http://www.rcsb.org/pdb/). The compound diethyl phthalate identified by GC-MS analysis was screened against the target protein. The compound details were retrieved from the Pubchem database, and the chemical structure was generated from SMILES notation by using the Chemsketch software (http://www.acdlabs.com). Autodock 4.2 version, the most common and freely available software, (http://autodock.scripps.edu) was used for the docking analysis. Lig Plot software (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) was used to study the interaction between ligand and protein.

RESULT AND DISCUSSION

The secondary metabolite from an endophytic fungus of *Aspergillus* sp. from *L. coramendalica* was analyzed in GC-MS. From the list

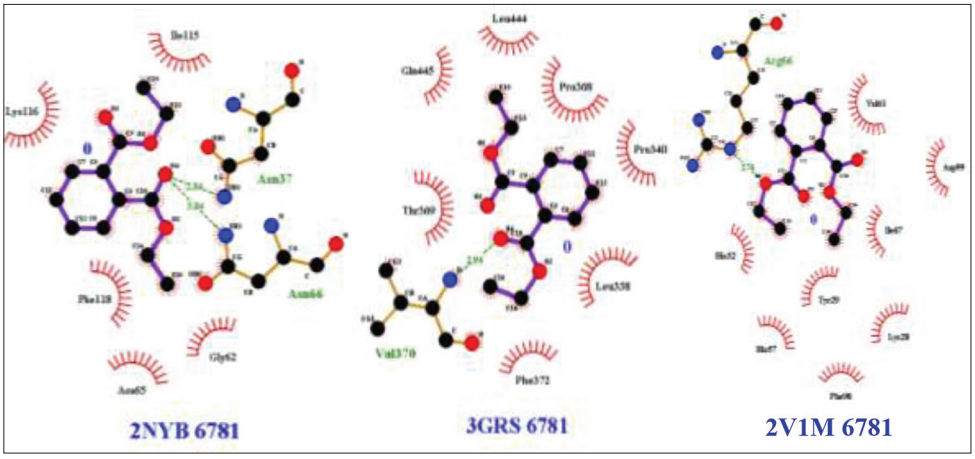

Fig. 1: Docking interaction of the protein and ligand and the predicted ligand binding site residues

Table 1: Docking energy of diethyl phthalate against antioxidant enzymes

Target protein	Ligand name	Docking energy level (kcal/mol)
SOD (2NYB)	Diethyl phthalate	−6.45
GSH reductase (3GRS)	Diethyl phthalate	−5.33
GPX (2V1M)	Diethyl phthalate	−4.46

SOD: Superoxide dismutase, GPX: Glutathione peroxidase, GSH: Glutathione reductase

of metabolites obtained those which possess antimicrobial and significant biological activity is thoroughly studied and identified as Diethyl phthalate. Diethyl phthalate is widely used as a plasticizer and softener, pharmaceutical coatings, cosmetic additives and also as an insecticide [12]. SOD's, catalases and GPXs are some of the enzymes which catalyze dismutation of superoxide free radicals.

When there is an increase in the dose concentration of the diethyl phthalate there is a considerable decrease in the activity of GPX, superoxide dismutase and GSH reductase. This increases our interest to find the interaction and the inhibitory action of the secondary metabolite on these proteins (enzymes). The present work provides support for a possible lethal pathway from antimicrobial treatment to free radical accumulation via superoxides and peroxides.

Autodock software was used to dock diethyl phthalate against antioxidant enzymes. The docking interaction of the protein and ligand and the predicted ligand binding site residues are shown in Fig. 1. The docked ligand molecules were selected based on docking energy and good interaction with active site residues, and the result is shown in Table 1.

Out of the 3 target protein SOD was the most potent having the least docking energy of −6.45 kcal/mol and the interaction shows two hydrogen bond with Asn37 and Asn66 and nonbonding interactions with Gly62, Asn65, Phe118, Lys116, Ile115. Lesser the binding score more the binding capacity of the ligand.

Whereas GSH reductase interaction studies show one hydrogen bond with Val 370 and non-bonding interactions with Thr369, Gln445, Leu444, Pro 368, Pro340, Leu38, Phe372 and the docking energy of −5.33 kcal/mol. GPX showed the docking energy of −4.46 kcal/mol having 1 H bond with Arg66 and non-bonding interactions withVal61, Asp99, Ile67, His32, Lys28, Phe98.

From the interaction studies it has been found that diethyl phthalate inhibits the activity of SOD, GPX, GSH reductase One possible explanation for the mechanisms is that oxidative diethyl Phthalate can antagonize metal ions (such as Cu^{2+}, Zn^{2+}, Fe^{2+}, Mg^{2+}) that are essential for the activity of antioxidant enzymes, resulting in a loss or decrease of the activities of SOD. As a result three major ROS, superoxide, H_2O_2, and •OH, are generated. The accumulation of superoxide anions following exposure to diethyl phthalate causes oxidative stress. Oxidative stress causes damages to nucleic acids, lipids and proteins in cells. Diethyl phthalate may induce cells to produce superoxide anion $•O_2$ and H_2O_2 among others; this results in an accumulation of the lipid peroxides, attacking the polyunsaturated fatty acid in the cell membranes of the organisms leading to cell death.

CONCLUSION

The present study shows that diethyl phthalate could be used as a potent antimicrobial agent. Further exploration of the function of the compound will facilitate a better understanding toward developing diethyl phthalate as an antimicrobial agent.

REFERENCES

1. Tan RX, Zou WX. Endophytes: A rich source of functional metabolites. Nat Prod Rep 2001;18(4):448-59.
2. Vining LC. Functions of secondary metabolites. Annu Rev Microbiol 1990;44:395-427.
3. Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, *et al*. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 2010;165(6):437-49.
4. Keren I, Shah D, Spoering A, Kaldalu N, Lewis K. Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. J Bacteriol 2004;186(24):8172-80.
5. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. A common mechanism of cellular death induced by bactericidal antibiotics. Cell 2007;130(5):797-810.
6. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. Cell 2008;135(4):679-90.
7. Fridovich I. Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol 1983;23:239-57.
8. Hassan HM, Fridovich I. Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. Arch Biochem Biophys 1979;196(2):385-95.
9. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335-44.
10. Apel K, Hirt H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004;55:373-99.
11. Barbehenn RV. Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. J Chem Ecol 2002;28(7):1329-47.
12. Page BD, Lacroix GM. The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985-1989: A survey. Food Addit Contam 1995;12(1):129-51.