Docking Analysis of Quercetin, Gallic acid and Hexanoic acid with seven Domains of Polyketide Synthase, A computational approach to Contemplate Quercetin, Gallic acid- mediated aflatoxin biosynthesis inhibition in *Aspergillus flavus*, A potent carcinogenic Fungus

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Submitted

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"The reward of the young scientist is the emotional thrill of being the first person in the history of the world to see something or to understand something, nothing can be compared with that experience"

AS WE DO MORE, WE LEARN MORE AND ULTIMATELY BECOME MORE.

Presentation, inspiration and motivation have always played a key role in the success of any venture. This journey would never have been completed without the support, well wishes and encouragement of many people. We would like to acknowledge all those who contributed to this project and helped us to reach this desired destination.

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Coming Together is a beginning, Staying together is Progress and Working together as a **TEAM** is **SUCCESS** .Unity is strength, when there is teamwork and collaboration, wonderful things can be achieved.

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CERTIFICATE

STUDENT DECLARATION

I hereby declare that the work presented in this Report Thesis entitled ***"Docking Analysis of Quercetin , Gallic acid and Hexanoic acid with seven Domains of Polyketide Synthase , A computational approach to Contemplate Quercetin, Gallic acid - mediated aflatoxin biosynthesis inhibition in Aspergillus flavu , A potent carcinogenic Fungus"*** in fulfillment of the requirements for the award of the Degree of Bachelor of Technology in Bioinformatics submitted in the Department of Biotechnology and Bioinformatics, Jaypee University Of Information Technology, Waknaghat , Himachal Pradesh, is an original record of my own work carried out over a period from July 2020 to May 2021 under the supervision of Dr. Jata Shankar (Associate Professor , Department of biotechnology and bioinformatics).

The matter expressed in the report has not been submitted for the award of any other degree or diploma.

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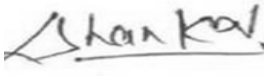
This is to certify that the above statement made by the candidates is true to the best of my knowledge.



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CHAPTER 1:

Docking analysis of Quercetin, Gallic acid and Hexanoic acid with seven domains of Polyketide Synthase, A computational approach to contemplate Quercetin, Gallic acid - mediated aflatoxin biosynthesis inhibition in *Aspergillus flavus*, A potent carcinogenic fungus

1.1 ABSTRACT

Aspergillus Flavus is the major opportunistic contaminant known to produce aflatoxin in food crops . Food contamination due to aflatoxins is a safety concern for agricultural yields[1]. In order to identify and develop novel anti-aflatoxigenic agents, Explorative Studies on Phytochemicals as anti-aflatoxigenic agents have accrued remarkable importance including Quercetin and Gallic acid which plays a significant role in providing defense mechanisms against it. The previously conducted experimental studies on Gallic acid and Quercetin suggested potential Inhibitor [2] .Thus, to understand the molecular mechanism behind inhibition of aflatoxin biosynthesis by Quercetin and Gallic acid , an interactive study with Polyketide Synthase A (PksA) of *Aspergillus flavus* was à fond conducted [3]. The 3D structure of PksA comprising of seven structural domains were modeled using SWISS-MODEL server and systematic Docking studies were performed using Auto dock tools-1.5.6 .Docking energies of the ligands Gallic acid , Quercetin and Hexanoic acid (as precursor) were compared with each of the domains of PksA enzyme. Binding energy for Quercetin and Gallic acid was lesser that ranged from (-6.96 to -5.86kcal/mol) and (-6.09 to -4.79 kcal/mol) in comparison to Hexanoic acid (-5.05 to -3.36 kcal/mol). LigPlot analysis showed the formation of bonds in case of quercetin, 7 H bonds in Gallic acid and 3 H bonds in Hexanoic acid .During an interaction with acyl transferase domain, both Gallic acid and Hexanoic acid showed H bond formation at Glu36, Arg8,Thr11 positions. Also, in PP domain, Gallic acid creates six H bonds in comparison to one in Hexanoic acid .In ACP, TH, KS, PT Domains , No. of H bonds were

observed more as compared to Gallic acid and Hexanoic acid. In AT, PP, CT domains, Gallic acid was observed to have more H bonds than Quercetin and Hexanoic acid.The dynamics of protein-ligand complex formation for every domain was investigated through MD simulations. Phytochemicals showed stable binding with active site of polyketide synthase A (PksA) indicated by steady RMSD of protein backbone atoms and potential energy profiles. Overall results revealed that Quercetin Followed by Gallic acid exhibited the highest level of binding potential (more number of H bonds) with PksA domain in comparison to Hexanoic acid; thus, Quercetin and Gallic acid feasibly inhibits by virtue of competitively binding to the seven domains of polyketide synthase, a fundamental enzyme of aflatoxin biosynthetic pathway. Further, we suggest that key enzymes from aflatoxin biosynthetic pathway in aflatoxin-generating Aspergilli could be traversed further using other phytochemicals as potential inhibitors.

1.2 INTRODUCTION

The project entitled **"Docking analysis of Quercetin , Gallic acid and Hexanoic acid with seven domains of Polyketide Synthase , A provided insight into Quercetin , Gallic acid - mediated aflatoxin biosynthesis inhibition in Aspergillus flavus"** is mainly concerned with objective of " STUDY ON PHYTOCHEMICALS AS ANTI-AFLATOXIGENIC AGENTS , COMPUTATIONAL APPROACH TO STUDY THE MOLECULAR MECHANISM BEHIND INHIBITION OF AFLATOXIN BIOSYNTHESIS BY GALLIC ACID, QUERCETIN AND HEXANOIC ACID AND STUDY THEIR INTERACTIONS WITH POLYKETIDE SYNTHASE PKSA OF *ASPERGILLUS FLAVUS*".

Aspergillus is indeed a morphological genus composed of around 250 recognizable species. The aspergillum is distinguished by its distinct and unique spore-bearing structure. *Aspergillus flavus* originally belonged to Flavi's section. This section includes the most critically Significant aflatoxin

developing fungus like *A. flavus*. and *A. parasiticus*. In this segment, the less prevalent aflatoxin producing fungus include *A. nomius*, *A. parviscleroti* genus, *A. bombysis* as well as *A. pseudotamarii* [4].

Aspergillus flavus is an avaricious pathogen for agricultural products. It is essential because in both pre and post harvest stages ,It causes aflatoxin to form as a secondary metabolite in a variety of crops [5].Aflatoxins are polyketide derived secondary metabolites ,highly regulated ,poisonous and active hepato-carcinogenic agents which are hazardous to animals as well as in humans, it is conventionally an exploitative pathogen for immuno-compromised individuals[6].

The development of aflatoxin is the combined effect of fungal life forms, substrates and the surrounding environment. The factors influencing the development of aflatoxin can be categorized into three parts: nutritional , biological and physical variables. Physical parameters like pH, relative humidity as well as moisture, light, aeration, can have an impact on aflatoxin production [7]. The genes involved in aflatoxin biosynthetic pathway are clustered, such as those of other secondary metabolites. Acetate' polyketides' are the primary precursors of the secondary metabolites. The diverse aflatoxins are developed through acetate as well as malonate building blocks formed mostly during idiophase. The four major key aflatoxins are CI7 compounds (AFB' AFB2, AFGI, and AFG2) defined as Nonaketides .Biosynthesis of aflatoxin has indeed been speculated to include at minimum 23 enzymatic processes. Aflatoxins are developed via Polyketide 3anthraquinones3xanthones3aflatoxins acetate 3 conversion pathways. At least 15 apparent structurally aflatoxin moieties in the aflatoxin biosynthetic pathway have thus far been established. It's been asserted that 25 genes are involved in aflatoxin biosynthesis and are clustered in a 70-kb segment of dna around the chromosome [8].

The polyketide biosynthetic pathway causes Aflatoxin biosynthesis in *A. flavus* to be a synchronous process. Polyketide synthase (PKS) is among the essential enzymes in the polyketide biosynthetic pathway [9]. Fungi corresponding to Type I polyketide Synthase comprises extremely significant multifunctional protein which is 180–250 kDa structured with Diverse multiple domains. Ketoacyl Synthase , acyl carrier proteins and acyl transferase are the three major domain groups . There have been up to 30 genes and a substantial regulatory gene named aflR gene is involved into aflatoxin biosynthesis involving fatty acid synthases [10]. Research on *Aspergillus nidulans* indicated that the generation of secondary metabolite has since been completely rebuilt in mutants whenever mutant of fatty acid synthase itself was processed with hexanoic acid as a precursor [11].

Polyketides are believed to be the prime recipients for the development of anti-contamination therapeutics in agricultural yields caused by aflatoxin. Polyketides have been identified to be efficacious in decreasing aflatoxin contamination, but there are certain limitations existing along with it [12].An alternative approach to fungal contamination is the interface of Phytochemicals synthesized from diverse plant sources[13]. Plants develop these secondary metabolites to provide a defense mechanism against pathogenic fungus[14].Due to the antimicrobial properties ,they have the capacity to protect humans and animals against certain diseases induced by microorganisms and even toxins linked with them[15]. For potential drug research and growth, these metabolites have become the most effective chemo-preventative compounds[16].

Specific biological polyphenols including Gallic acid and Quercetin exhibits optimum inhibition against AFB1 production in *Aspergillus flavus*[17]. In almost every part of the plant, such as bark,

wood, leaf, fruit, root as well as seed, Gallic acid and its derivatives are found in natural environment. Since using natural plant products and bio-control additives instead of synthetic chemicals allows scientists to avoid using toxic preservatives, this substitute to synthetic drugs is becoming a primary concern for scientists all over the world.Thus, to understand the Quercetin , Gallic acid and PksA interaction, Computational methodology could be a valuable tool for researching aflatoxin inhibition mechanisms. The protein sequence of PksA from *A. flavus* was extracted from UniProt database to execute homology modeling. Phytochemicals has been docked with the various PksA domains of *A. Flavus*, and as compared to the substrate, Hexanoic acid, it binds more efficiently. The position of H bonding and hydrophobic interaction of Quercetin ,Gallic acid and Hexanoic acid with seven distinct domains of PksA was compared using Ligplot analysis. The dynamics of protein-ligand complex formation for every domain was investigate through MD simulations. In contrast to Hexanoic acid, our results indicated that Quercetin and Gallic acid had the highest degree of binding potential with PksA; therefore, Quercetin then Gallic acid could inhibit by conservatively binding to the seven domains of Polyketide Synthase, a key enzyme in the aflatoxin biosynthetic pathway.

Aflatoxins are responsible for approximately to 25% of the entire world food crops being harmed, leading to economic losses in developed nations as well as human and animal illness in developing ones[18].Pathogenic and opportunistic fungal infections found in Aspergillus flavus species have the potential to induce mycosis (invasive aspergillosis) in humans. The emergence of drug-resistant isolates has limited the effectiveness of current antifungal medicines[19]. Various adapted cultural methods are now being used and researched to combat the problem of food contamination[17].Our study aim to evaluate the effect of phytochemicals like gallic acid as an alternative approach to

fungal contamination and it may find its application in Phytochemicals based anti- aflatoxigenic agents.

1.3 LITERATURE REVIEW

1.3.1 INTRODUCTION

By producing aflatoxin (AFB1 and AFB2), *Aspergillus flavus* is one of the most potent pollutants of raw food commodities during pre- and post-harvest crops. The International Agency for Research on Cancer classifies these toxins as Category I toxins because they cause cancer. To prevent the toxin's severe effects on humans and animals, different safety administrations control the toxin's exposure level. A. flavus is also one of the most common causes of aspergillosis in immunocompromised patients. Aflatoxin biosynthesis in *A. flavus* is a multi-step process involving the polyketide biosynthetic pathway. Is one of the most important enzymes in the polyketide biosynthesis pathway?

1.3.2 *ASPERGILLUS FLAVUS*, A POTENT CARCINOGENIC FUNGUS

Aspergillus is a morphological genus that has over 250 species .The aspergillum is distinguished by its peculiar and exclusive spore-bearing structure. Aspergillus flavus was previously mentioned in Flavi's part. The highest deadly aflatoxin-producing fungus, such as *A. flavus* and *A. parasiticus* [4], are included in this category. Aspergillus flavus is a hazardous fungus that destroys agricultural crops It's significant since it generates aflatoxin to develop as a secondary metabolite in a number of crop types before and after harvest. It's an uncontrolled agricultural pathogen. It's critical to establish aflatoxin as a secondary metabolite in a range of crops either before and after harvest. Aflatoxin is a harmful carcinogen that is strictly controlled throughout most nations. Aflatoxin has

been related to drought-stressed oilseed crops in the zone, including maize, peanuts, cottonseed, and tree nuts [20]. Under the right circumstances the fungus may grow and generate aflatoxin in nearly any preserved crop seed. Aflatoxin can be stored safely if the moisture level is kept below the minimum necessary for *A. flavus* development. Improving cultural methods, creating resilient crops using molecular and proteomic approaches, selective elimination by non-producing strains, and creating field treatments that prevent aflatoxin production are all being utilized or explored.

1.3.2.1 HOST RANGE OF ASPERGILLUS FLAVUS

Aspergillus flavus has a wide habitat spectrum as an uncontrolled pathogen/saprobe. This kind of soil fungus is quite prevalent. The main problem with this fungus in agriculture is that it develops extremely carcinogenic chemicals known as aflatoxins, which are detrimental to the welfare of animals. In the region, *A. flavus* is mostly a problem in the oilseed crops maize, peanuts, cottonseed, and tree nuts. Under the incorrect storage circumstances, A. flavus may develop and generate aflatoxin in nearly every crop seed. Animals and insects are both affected by this disease. In immunocompromised individuals, it is predominantly an uncontrolled pathogen.

1.3.2.2 BIOLOGY AND HABITAT OF A. FLAVUS

Aspergillus flavus species can pollute a variety of agricultural resources in field preservation sites comprising processing plants, and throughout transit. So far, af1atoxins have exclusively been discovered in *A. flavus, A. parasiticus,* and *A. nomius* [21].Toxicity of *A. flavus* strains ranges from harmless to generating aflatoxins B1 and B2, whilst aflatoxins BI', B2, GI, and G2 are produced by *A. flavus, A. parasiticus*. Aflatoxin-producing fungus are all soil microorganisms, although their dispersion patterns differ [22].In moderate climates *A. flavus* spores can be discovered in the air

rather than in the soil. *A. flavus*, *A. parasiticus* thrives in hotter temperatures like the tropics and subtropics, and has been linked to soil [[23].

1.3.3 AFLATOXINS

After more than 100,000 turkey poults in England died of apparent poisoning from mold-contaminated peanut meal in the early 1960s, aflatoxins were discovered and characterized [24]. There are two types of aflatoxicosis, a disease caused by aflatoxin toxicity. Acute aflatoxicosis can be fatal. Cancer, with the liver as the primary target organ, immune suppression, teratogenicity, and other symptoms are all symptoms of chronic aflatoxicosis. Aflatoxins are difuranocoumarin derivatives that are formed via a polyketide pathway. The characters relate to the color of their fluorescence underneath ultraviolet light (blue or green), and the numbers indicate their relative migration distance on a thin-layer chromatographic plate. Only four significant aflatoxins, B1, B2, G1, and G2 (AFB1, AFG1, AFB2, and AFG2), contaminate crops and potentially cause risk to livestock and human health, out of at least 16 structurally related aflatoxins identified. The most hepatocarcinogenic naturally produced aflatoxin is Aflatoxin B1 which is a cancer-causing substance [25].*A. flavus* produces aflatoxins, which are the most toxic and active hepatocarcinogens ever discovered in nature. Due to contrasting morphological and biochemical attributes, reliable species identification within the Aspergillus flavus complex remains difficult, and more taxonomic and population genetics work is needed to better understand the species and related species. The most toxic and carcinogenic compounds are aflatoxins, a category of polyketide-derived furanocoumarins. AFB1 and AFB2 are produced by *Aspergillus flavus*.

1.3.4 Polyketide synthase (Pks)

PKS is a monomeric protein that utilizes acyl units to generate complex natural products. Type I polyketide synthase is a very broad versatile protein (180–250 kDa) with several domains found in fungi. The key domain types are ketoacyl synthase (KS), acyl transferase (AT), and acyl carrier proteins (ACP).

1.3.5 DOMAINS OF PROTEIN

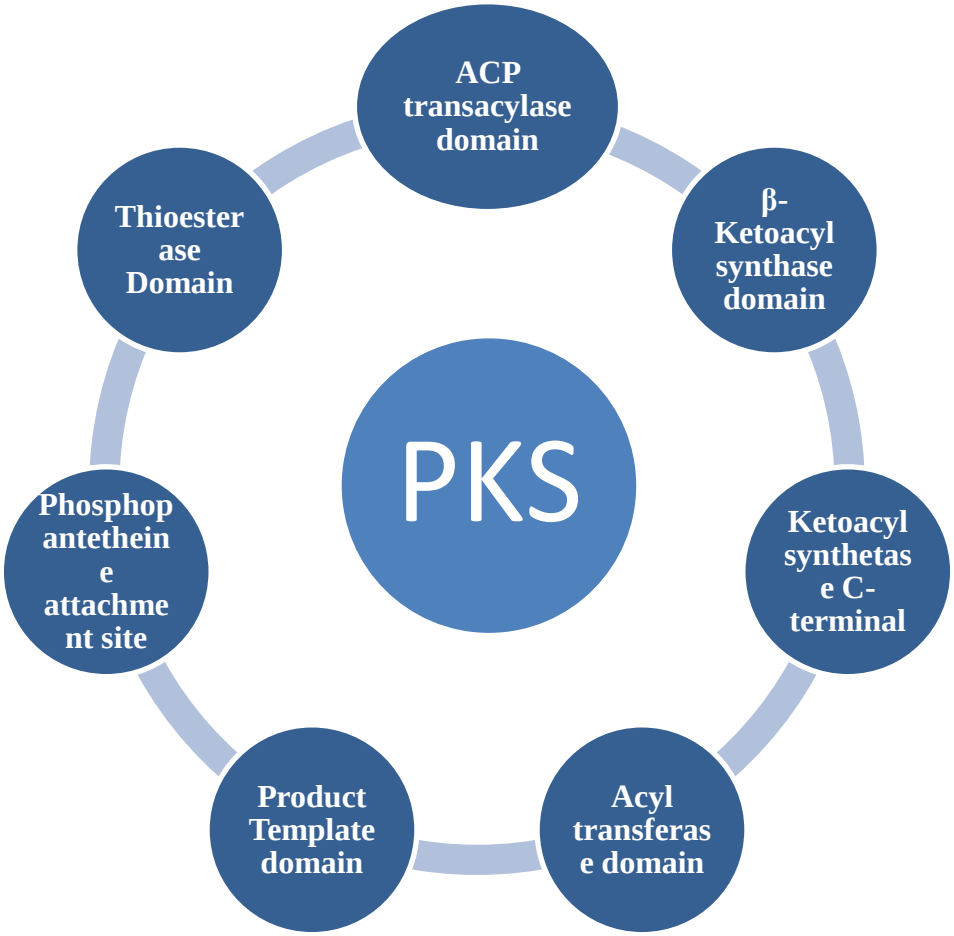

FIGURE 2 : SEVEN DOMAINS OF POLYKETIDE SYNTHASE A

1.3.6 STRUCTURE OF PROTEIN

Parameters used for protein structural assessment by PROCHECK analysis are described in the following table.

DOMAINS	**RAMACHANDRAN PLOT(%)**	**G FACTOR**	**MCBL(%)**	**RESIDUES**	**OVERALL QUALITY FACTOR**
ACP transacylase domain	91.9[a]; 6.6[b]; 0.0[c]; 1.4[d]	-0.08[e] ; 0.15[f]; 0.03[g]	91.9	238	93.33
β-Ketoacyl synthase domain	88.3[a];10.3[b];1.1[c];0.3[d]	-0.25[e] ; 0.16[f] ; -0.07[g]	88.3	429	89.31
Ketoacyl synthetase C-terminal domain	85.3[a]; 13.2[b];1.5[c];0.0[d]	-0.27[e] ; 0.13[f] ; -0.10[g]	85.3	79	63.04
Acyl transferase domain	94.3[a] ;5.3[b] ;0.0[c] ; 0.4[d]	-0.15[e] ; 0.14[f] ; -0.02[g]	94.3	299	97.53
Product template domain	89.3[a] ;10.0[b] ;0.7[c] ;0.0[d]	-0.31[e] ;0.21[f] ; -0.09[g]	89.3	327	88.21

Phosphopantetheine attachment site	86.7[a] ;13.3[b] ;0.0[c] ;0.0[d]	-0.40[e] ; -0.01[f] ; -0.24[g]	86.7	68	95.91
Thioesterase domain	90.4[a] ;9.1[b] ;0.5[c] ;0.0[d]	-0.25[e] ; 0.19[f] ; -0.06[g]	90.4	239	95.51

MCBL distribution of main chain bond length , Overall quality factor by ERRAT

a Residue in favored regions

b Residue in allowed regions

c Residue in generously allowed regions

d Residue in disallowed regions

e *G* factor score of dihedral bond

f G factor score of covalent bond

g Overall *G* factor score

1.3.7 PHYTOCHEMICALS AS INHIBITORS

Plants generate phytochemicals to assist them to withstand diseases from fungus, bacteria, and plant viruses, as well as for ingestion by insects and certain other animals. The term phyton means 'plant' in Greek. These have biological action in the plant habitat and aid in plant development as well as defense versus diseases and competition.

Secondary metabolites are generated by plants as a protection against pathogenic microorganisms, insects, and harsh environmental conditions. These non-nutritive metabolites are referred to as phytochemicals [26]. However, because of their antimicrobial properties, they can safeguard humans and animals from diseases induced by micro-organisms or toxins correlated with them[13].

For potential drug research and growth, metabolites are the most effective chemo-preventative agents[27]. There are several large classes of phytochemical compounds that have been discovered to date, each with its chemical structure[28].

Phenolic compounds, phytosterols, carotenoids, tools, terpenoids, alkaloids, flavonoids, saponins, tannins, aromatic acids, glucosinolates, carotenoids, essential oils, chlorophyll, and organic acids, along with proteases inhibitors, are the primary categories of phytochemicals [29]. Such chemicals can operate directly or indirectly to prevent illnesses or fungal infections because some include antimutagenic, antigenotoxic, antimicrobial, anthelmintic, anticarcinogenic, antiproliferative, anti-inflammatory, and antioxidant capabilities[30].

Biologically produced polyphenols including Gallic acid, catechin, epigallocatechin, and Quercetin, of which Quercetin and Gallic acid inhibited AFB1 synthesis in A. flavus the most. The use of phytochemicals derived from a variety of plant sources might be a viable option for treating fungal infestation [31].

1.3.7.1 GALLIC ACID

Gallic acid is a phenolic acid found in a diverse range of relatively high plant species both within its pure form and as a component of greater complex compounds like ester derivatives or polymers. Gallic acid and its metabolites may be found in almost every component of a plant, including the bark, wood, leaf, fruit, root, and seed. They might very well be discovered in a range of foods, notably blueberries, blackberries, strawberries, plums, grapes, mango, cashew nut, hazelnut, walnut, tea, and wine. Gallic acid (GA), a component of the Tulare walnut pellicle, has shown to be a

potent inhibitor of aflatoxin biosynthesis [32], GA was proven to greatly inhibit the expression of the farB gene, which regulates peroxisomal fatty acid oxidation[33], as well as the carbon repression regulator encoding gene, creA, which was recently discovered to be engaged in aflatoxin production[34]. Simultaneously, 0.8 percent (w/v) GA treatment significantly reduced the expression of about all allocated genes in the aflatoxin biosynthesis cluster. This sheds light on the biological processes underlying oxidative stress responses in A. flavus that contribute to aflatoxin biosynthesis.

1.3.7.2 QUERCETIN

Quercetin is a plant polyphenol that belongs to flavonoids, commonly found in fruit, vegetables, seeds, tea, wine, flower, nuts, propolis, and honey. To remove aflatoxin from the food chain, a long-term and cost-effective source is needed. Natural Phytochemicals derived from various parts of plants, including fruits, vegetables, and spices, have been shown to have a broad variety of biological effects, including antioxidant, antimicrobial, and anti-inflammatory properties. As a result, to control contamination and increase yield, alternative approaches that are both economically feasible and environmentally sustainable are needed [35]. The inhibition of AFB1 development in A. flavus by Quercetin produced promising results[36], including aflatoxin inhibition at 800 g/mL. In aflatoxin-mediated hepatic harm cells (HepG2) in mice, Quercetin inhibited the formation of reactive oxygen species, cytotoxicity, and lipid per oxidation[37]. To learn more about quercetin's function in inhibiting aflatoxin biosynthesis in A. flavus, researchers conducted a study where they used nano-liquid chromatography-quadruple time-of-flight mass spectrometry to analyze the proteome of *Aspergillus flavus* cultured in corn flour media (CF) and corn flour media with Quercetin (nLC-Q-TOF). It showed that in contrast to CF, trans-membrane transporter proteins were highly expressed in response to CFQ. In addition, in contrast to CF, the

cAMP/PKA signaling pathway was observed in CFQ. AFB1 was also measured using quantitative high-performance liquid chromatography (HPLC) at various time points (7, 12, 24, and 48 hours). Overall, it showed that the presence of Quercetin inhibited aflatoxin biosynthesis in *A. flavus* [1].

1.3.8 ABOUT INHIBITION OF AFLATOXIN BIOSYNTHESIS

The natural occurrence, diagnosis, classification, biosynthesis, and genetic regulation of aflatoxins, and also the prevention and management of aflatoxin contamination of food and feed, have all been studied in great depth. At least 23 enzymatic reactions are thought to be involved in the biosynthesis of aflatoxin. In the aflatoxin biosynthetic pathway, at least 15 structurally aflatoxin intermediates were identified so far. It has been shown that 25 genes clustered within a 70-kb DNA region on the chromosome play a role in aflatoxin biosynthesis. Aflatoxin biosynthesis genes, including those for certain secondary metabolites, are grouped [38]. The cluster has been sequenced and annotated in its entirety[39]. Understanding the biosynthesis of aflatoxin is hoped to aid in the creation of control methods as well as provide insight into how and why aflatoxin developed. In A. flavus, there are around 30 genes in aflatoxin synthesis, including fatty acid synthases, as well as a major regulatory gene (AflR). When mutations of fatty acid Synthase were encountered with Hexanoic acid, the synthesis of secondary metabolites was preserved in mutants, according to a study on *Aspergillus nidulans*. It was suggested that polyketides are the frontline for the development of therapeutics against aflatoxin contamination in various agricultural crops[40]. The application of the biological agent to out-compete toxic effects of aflatoxins in pre- and post-harvested food crops has proven to be effective in reducing aflatoxin contamination; however, certain limitations exist.

1.3.9 COMPUTATIONAL APPROACH TO CONTEMPLATE STUDY

Understanding the mechanism of aflatoxin inhibition could be aided by a computational approach. As a result, developing a novel target necessitates an understanding of the host's mRNA and protein expression systems in response to stress. To best explain the interaction between Quercetin, Gallic

acid, and PksA, the protein sequence of PksA from A. flavus was evaluated. Furthermore, utilizing Quercetin and Gallic acid as ligands and Hexanoic acid as a precursor, domains and molecular docking studies were carried out. The total findings of molecular docking and LigPlot analysis, which included binding energy, electrostatic energy, H bonding, bond length, and hydrophobic interaction, suggested that Quercetin, Gallic acid, had the most binding potential with PksA domains in comparison to Hexanoic acid.

1.3.10 SOFTWARES USED

1.4 MATERIALS AND METHODS

1.4.1 ANALYSIS OF BIOLOGICAL DATA AND SEQUENCE RETRIEVAL

The complete amino acid sequence of A. flavus polyketide Synthase was acquired from the protein database of NCBI (National Centre For Biological Information), with the NCBI gene ID of 7914331, gene symbol: AFLA_139410 , gene description: **AflC / pksA / pksL1 / polyketide Synthase** and Uniprot accession number B8NI04_ASPFN. FASTA SEQUENCE was retrieved from the NCBI database.

FASTA

aflC / pksA / pksL1 / polyketide synthase [Aspergillus flavus NRRL3357]

NCBI Reference Sequence: XP_002379951.1

GenPept Identical Proteins Graphics

```
>XP_002379951.1 aflC / pksA / pksL1 / polyketide synthase [Aspergillus flavus NRRL3357]
MAQSRQLFLFGDQTADFVPKLRSLLSVQDSPILAAFLDQSHYVVRAQMLQSMNTIDHKLARTADLRQMVQ
KYVDGKLTPAFRTALVCLCQLGCFIREYEESGNMYPQPSDSYVLGFCMGSLAAVAVSCSRSLSELLPIAV
QTVLIAFRLGLCALEMRDRVDGCSDDRGDPWSTIVWGLDPQQARDQIEVFCQTTNAPQTRRPWISCISKN
AITLSGSPSTLRAFCEMPQMAQHRTALIPICLPAHNGALFTQADITTILDTTPTTPWEQLPGQIPYISHV
TGNVVQTTNYRDLIEVALSETLLEQVRLDLVETGLPRLLQSRQVKSVTIVPFLTRMNETMSNILPVSFIS
TETRTDTGRAIPASGRPGAGKCKLAIVSMSGRFPESPTTESFWDLLYKGLDVCKEVPRRRWDINTHVDPS
GKARNKGATKWGCWLDFSGEFDPRFFGISPKEAPQMDPAQRMALMSTYEAMERAGLVPDTTPSTQRDRIG
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VILDAKTNHSAMSESMTRPHVGAQIDNMTAALNTTGLHPNDFSYIEMHGTGTQVGDAVEMESVLSVFAPS
ETARKADQPLFVGSAKANVGHGEGVSGVTSLIKVLMMMQHDTIPPHCGIKPGSKINRNFPDLGARNVHIA
FEPKPWPRTHTPRRVLINNFSAAGGNTALILEDAPERHWPTEKDPRSSHIVALSAHVGASMKTNLERLHQ
YLLKNPHTDLAQLSYTTTARRWHYLHRVSVTGASVEEVTRKLEMAIQNGDGVSRPKSKPKILFAFTGQGS
QYATMGKQVYDAYPSFREDLEKFDRLAQSHGFPSFLHVCTSPKGDVEEMAPVVVQLAITCLQMALTNLMT
YFGIRADVTVGHSLGEFAALYAAGVLSASDVVYLVGQRAELLQERCQRGTHAMLAVKATPEALSQWIRDH
DCEVACINGPEDTVLSGTTKNVAEVQRAMTDNGIKCTLLKLPFAFHSAQVQPILDDFEALAQGATFAKPQ
LPILSPLLRTEIHEQGVVTPSYVAQHCRHTVDMAQALRSAREKGLIDDKTLVIELGPKPLISGMVKMTLG
DKISTLPTLAPNKAIWPSLQKILTSVYTGGWDINWKNYHAPFASSQKVVDLPSYGWDLKDYYIPYQGDWC
LHRHQQDCKCAAPGHEIKTADYQVPPESTPHRPSKLDPSKEAFPEIKTTTTLHRVVEETTKPLGATLVVE
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HGKGPQLLRTTLTMEWPPKAAATTRSAKVKFATYFADGKLDTEHASCTVRFTSDAQLKSLRRSVSEYKTH
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HVDAITQVAGFAMNANDNVDIEKQVVVNHGWDSFQIYQPLDNSKSYQVYTKMCQAKENDLVHGDVVVLDG
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ATHSHTPQQPKHSPVPETAGSAPAAKGVGVSNEKLDAVMRVVSEESGIALEELTDDSNFADMGIDSLSSM
VIGSRFREDLGLDLGPEFSLFIDCTTVRALEDFMLGSGDAGSGSNVEDPPPSATPAINPEIDWSSSASDS
IFASEDHGHSSESGADTGSPPALDLKPYCRPSTSVVLQGLPMVARKTLFMLPDGGGSAFSYASLPRLKSD
TAVVGLNCPYARDPENMNCTHGAMIESFCNEIRRRQPRGPYHLGGWSSGGAFAYVVAEALVNQGEEVHSL
IIIDAPIPQAMEQLPRAFYEHCNSIGLFATQPGASPDGSTEPPSYLIPHFIAVVDVMLDYKLAPLHARRM
PKVGIVWAADTVMDERDAPKMKGMHFMIQKRTEFGPDGWDTIMPGASFDIVRADGANHFTLMQKEHVSII
SDLIDRVMD
```

FIGURE 3 : FASTA SEQUENCE OF POLYKETIDE SYNTHASE

1.4.2 Protein Blast for finding Homologous sequences

The regions of similarity between the native sequence and other sequences were found using BLAST, The FASTA sequence was used as a QUERY to search for homologous sequences using BLAST in NCBI.

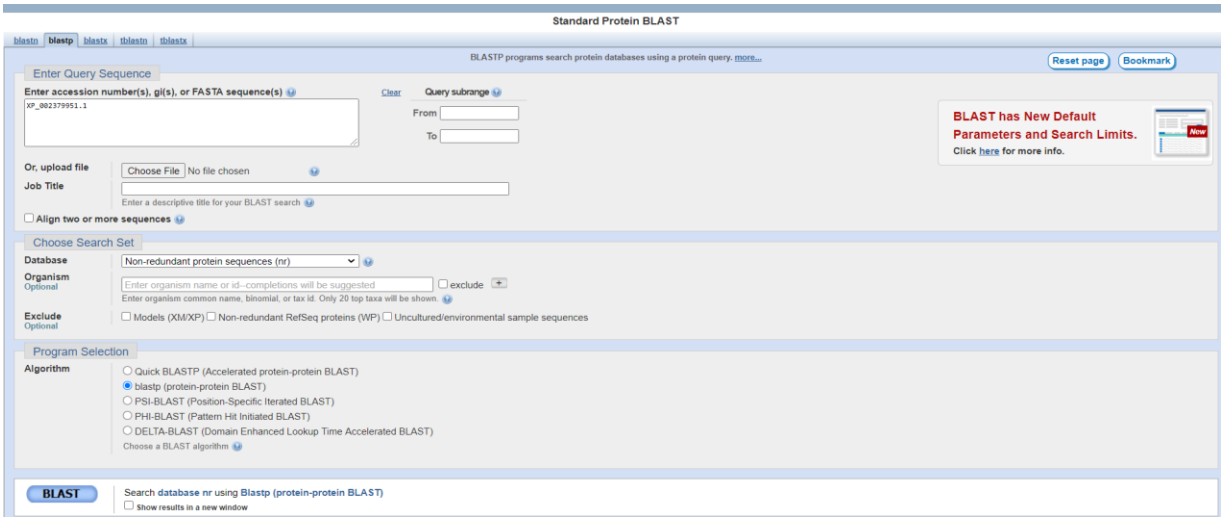
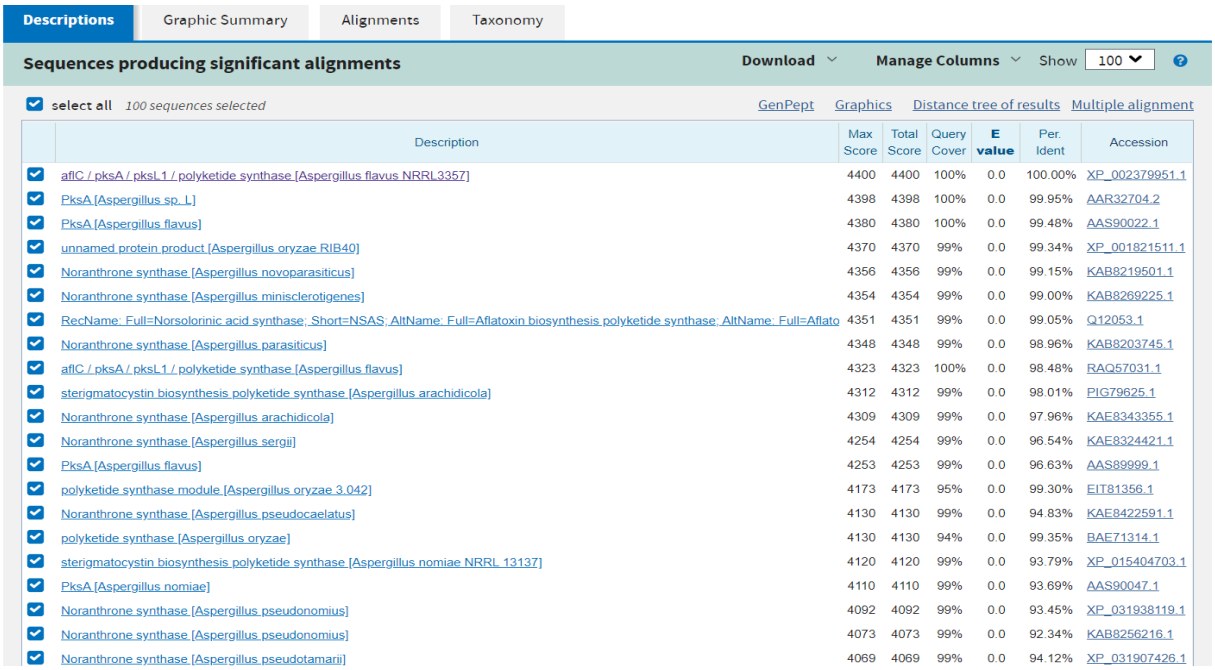


FIGURE 4 : PROTEIN BLAST

The homologous sequences of more than 90% sequence identity were retrieved.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Manage Columns — Show 100

select all *100 sequences selected* — GenPept — Graphics — Distance tree of results — Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
aflC / pksA / pksL1 / polyketide synthase [Aspergillus flavus NRRL3357]	4400	4400	100%	0.0	100.00%	XP_002379951.1
PksA [Aspergillus sp. L]	4398	4398	100%	0.0	99.95%	AAR32704.2
PksA [Aspergillus flavus]	4380	4380	100%	0.0	99.48%	AAS90022.1
unnamed protein product [Aspergillus oryzae RIB40]	4370	4370	99%	0.0	99.34%	XP_001821511.1
Noranthrone synthase [Aspergillus novoparasiticus]	4356	4356	99%	0.0	99.15%	KAB8219501.1
Noranthrone synthase [Aspergillus minisclerotigenes]	4354	4354	99%	0.0	99.00%	KAB8269225.1
RecName: Full=Norsolorinic acid synthase; Short=NSAS; AltName: Full=Aflatoxin biosynthesis polyketide synthase; AltName: Full=Aflato	4351	4351	99%	0.0	99.05%	Q12053.1
Noranthrone synthase [Aspergillus parasiticus]	4348	4348	99%	0.0	98.96%	KAB8203745.1
aflC / pksA / pksL1 / polyketide synthase [Aspergillus flavus]	4323	4323	100%	0.0	98.48%	RAQ57031.1
sterigmatocystin biosynthesis polyketide synthase [Aspergillus arachidicola]	4312	4312	99%	0.0	98.01%	PIG79625.1
Noranthrone synthase [Aspergillus arachidicola]	4309	4309	99%	0.0	97.96%	KAE8343355.1
Noranthrone synthase [Aspergillus sergii]	4254	4254	99%	0.0	96.54%	KAE8324421.1
PksA [Aspergillus flavus]	4253	4253	99%	0.0	96.63%	AAS89999.1
polyketide synthase module [Aspergillus oryzae 3.042]	4173	4173	95%	0.0	99.30%	EIT81356.1
Noranthrone synthase [Aspergillus pseudocaelatus]	4130	4130	99%	0.0	94.83%	KAE8422591.1
polyketide synthase [Aspergillus oryzae]	4130	4130	94%	0.0	99.35%	BAE71314.1
sterigmatocystin biosynthesis polyketide synthase [Aspergillus nomiae NRRL 13137]	4120	4120	99%	0.0	93.79%	XP_015404703.1
PksA [Aspergillus nomiae]	4110	4110	99%	0.0	93.69%	AAS90047.1
Noranthrone synthase [Aspergillus pseudonomius]	4092	4092	99%	0.0	93.45%	XP_031938119.1
Noranthrone synthase [Aspergillus pseudonomius]	4073	4073	99%	0.0	92.34%	KAB8256216.1
Noranthrone synthase [Aspergillus pseudotamarii]	4069	4069	99%	0.0	94.12%	XP_031907426.1

FIGURE 5 : HOMOLOGOUS SEQUENCES

1.4.3 Homology modeling and Phylogenetic analysis

A phylogenetic tree is a branching diagram that depicts the phylogenetic connection between diverse biological species based upon similarities and differences in their structure and function. Following the Maximum likelihood, the method provides probabilities of the sequences given a model of their evolution. The more probable the sequences are given in the tree, the more the tree is preferred. The homologous sequences with more than 90% identity were aligned using the MEGA 6.06 tool and as a result, a phylogenetic tree was constructed, using the maximum likelihood method.

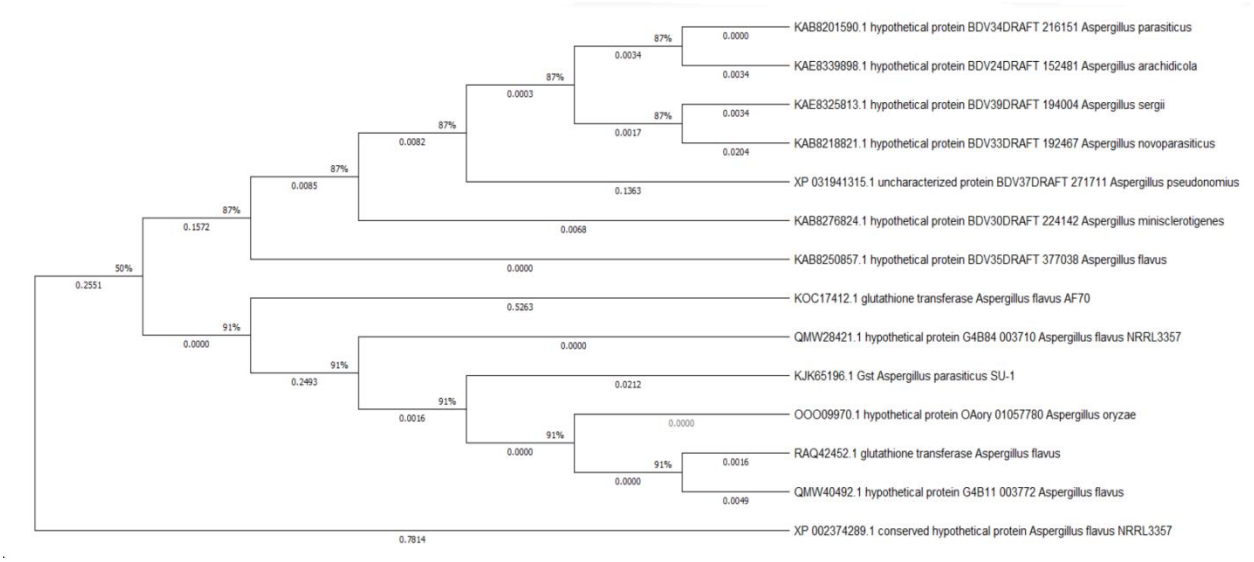


FIGURE 6: PHYLOGENETIC TREE OF HOMOLOGOUS SEQUENCES

1.4.4 LIGAND PREPARATION

Ligands must be prepared to create 3-D geometrics, assign proper bond orders, charges, hydrogens, and minimize the structure. Ligand preparation is done before docking for energy minimization as it

is used to reduce the overall potential energy of the ligand since biological systems are very dynamic and have low potential energies (-ve delta G) for Spontaneous interactions. Less is the energy, more spontaneous will be the interactions.

SOFTWARE AND DATABASE USED: PubChem and UCSF CHIMERA.

STEPS INVOLVED IN LIGAND PREPARATION :

1. Opened UCSF Chimera for VISUALIZATION and ANALYSIS of molecular structures, used for ligand preparation before docking.
2. Opened PubChem in the browser and type inhibitor (ligand) name i.e. GALLIC ACID and HEXANOIC ACID.
3. Opened PubChem in the browser and type inhibition (ligand) name i.e. GALLIC ACID and HEXANOIC ACID.
4. In UCSF Chimera Tools, go to Structure Editing followed by Build Structure. Paste the SMILES string that we have copied from PubChem and apply.
5. Now, the ligand molecule was built.
6. Opened Tools and did Structure Editing to Minimize Structure (here the steepest descent steps are set as 1000 and Conjugate gradient steps as 1000)
7. The Add hydrogens window poped-up, clicked OK, then Assigned Charges to minimize select Gasteiger and entered OK. This showed the net charge of the molecule. Clicked OK.Saved ligand to the working directory: saved as .mol2

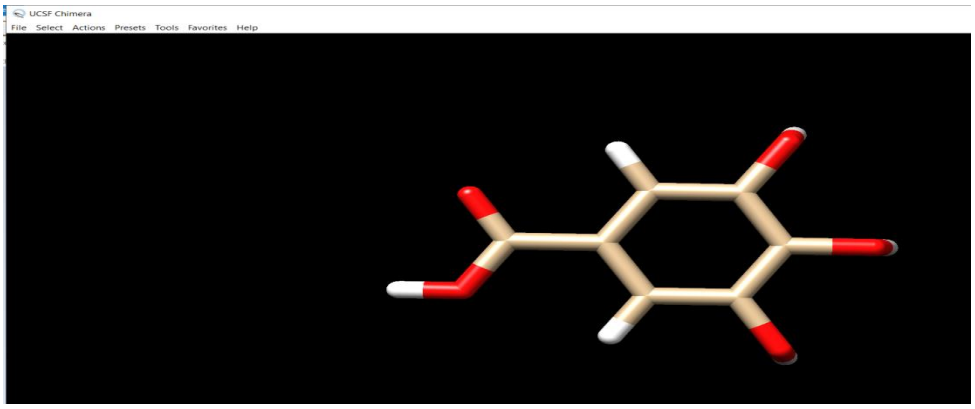
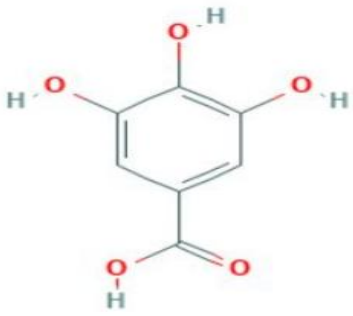


FIGURE 7: GALLIC ACID LIGAND VISUALIZATION IN PYMOL

1.4.5 Analysis of molecular properties of ligands

In the UCSF chimera, a three-dimensional structure of both ligands including Gallic acid and Hexanoic acid was developed, which was visualized in Pymol, and **Table 1** shows the results of Lipinski's five-rule evaluation. The results revealed drug-like attributes for these two ligands, along with three - dimensional structure for molecular docking research.



GALLIC ACID

HEXANOIC ACID

FIGURE 8: Three-dimensional structure of Gallic Acid (PubChem CID:370) and hexanoic acid (Id: 8892) were obtained from Pubchem

Compound	Molecular weight	H-bond donor	H-bond acceptor	Log P	Molecular Refractivity	Pubchem Cid	Molecular formula
Hexanoic acid	116.16g/mol	1	2	1.92	125.3074	8892	C6H12O2
Gallic acid	170.12g/mol	4	5	0.7	100.4250	370	C7H605

TABLE 1: Lipinski's rule of 5 outlines the molecular properties of ligands

1.4.6 PROTEIN STRUCTURE PREPARATION

SOFTWARE AND DATABASE USED: NCBI, SWISS MODEL, AND UCSF CHIMERA

1. Seven different domains of PksA from A.flavus with their respective locations were retrieved from the GENE database of NCBI.

2. Took the protein sequence from the UniProt knowledge database, extracted the desired located sequence from the whole protein sequence for each domain.

3. The SWISS-MODEL service was used to design all seven domains of PksA, and the best feasible model was chosen based on the sequence discovered for homology modeling. To locate templates

and align target templates, the SWISS-MODEL template library employs the BLAST and HHblits search engines.

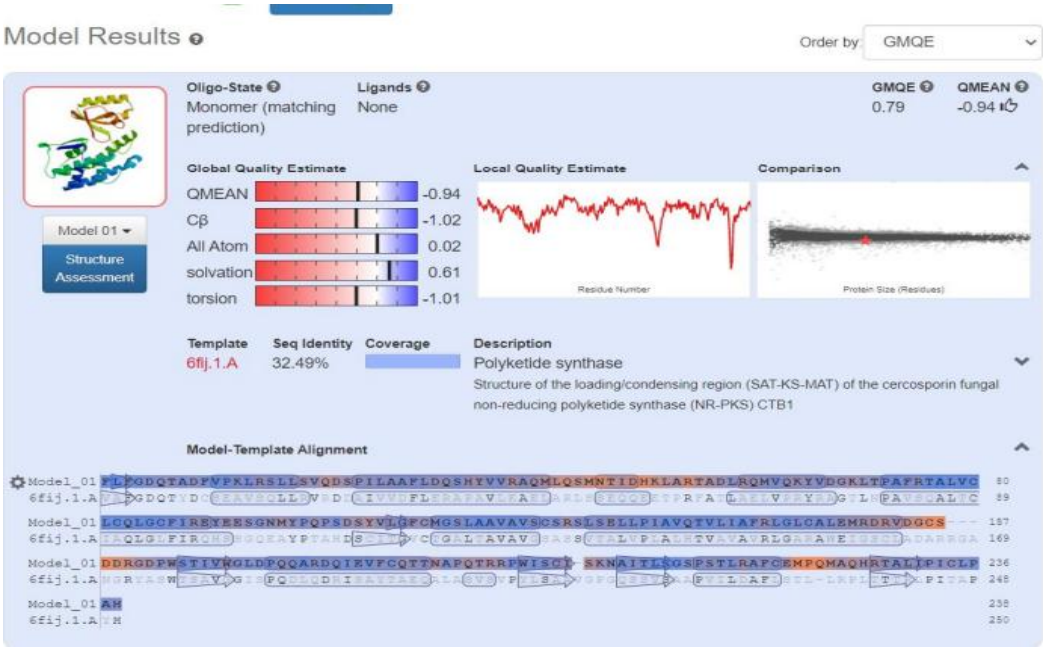


Figure 9 – Swiss model analysis

4. These methods ensure strong alignment at all levels of sequence identity, resulting in an experimental approach, resolution (if applicable), quaternary state assignment, sequence identity to the target, coverage, as well as other factors that point to the best possible template for our structure.

5. For further investigation, all protein structures are stored in PDB format.

6. Opened UCSF Chimera for VISUALIZATION and ANALYSIS of molecular structures of seven domains, used for protein preparation before docking.

7.Selected Tools -> Structure Editing -> Minimize Structure (here set steepest descent steps: 100 and Conjugate gradient steps:100) -> Minimize

8.Added hydrogens, then Assigned Charges to minimize select Gasteiger and enter OK. This will show the net charge of the molecule. Click OK.

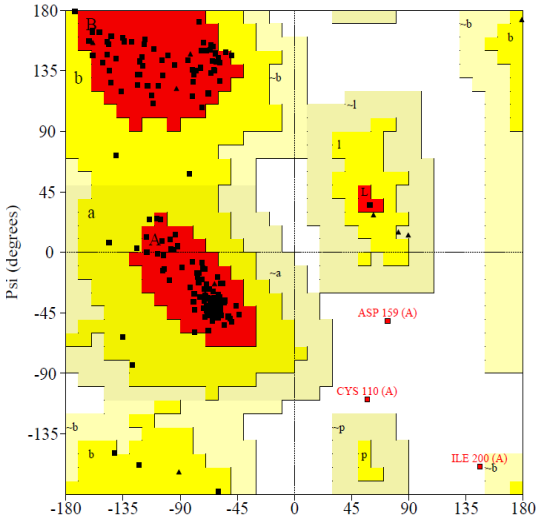
9. Saved the protein to the working directory: save as .pdb.

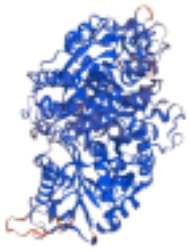
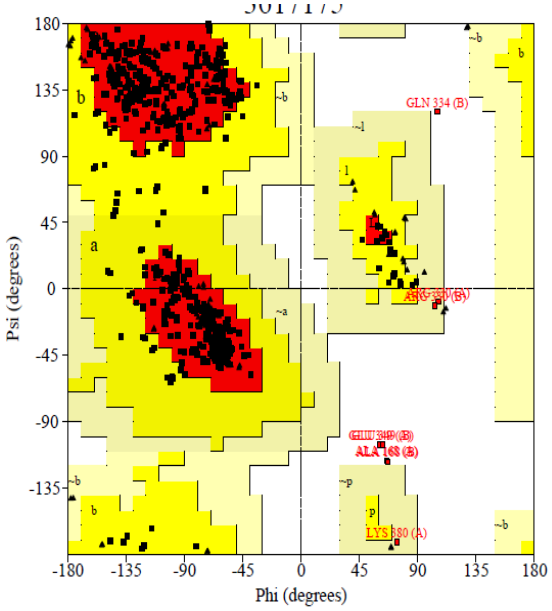
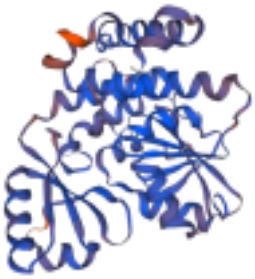
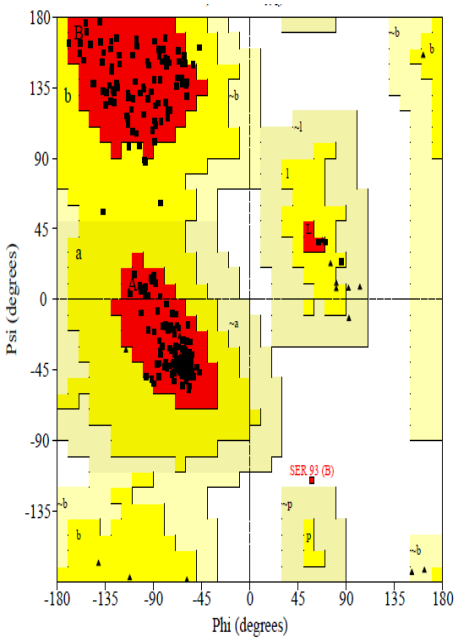
DOMAIN NAME	SMTL ID	SEQUENCE IDENTITY
ACP Trans acylase domain	6fij.1.A	32.49%
Beta-Ketoacyl Synthase	6fij.1.A	58.78%
Acyl Transferase Domain	6fik.1.B	39.60%
PKS Product Template Domain	3hrr.1.A	99.69%
Phosphopantetheine Attachment Site	2kr5.1.A	98.53%
Thioesterase Domain	3ils.1.A	99.58%
Ketoacyl-synthetase C-terminal extension	6fij.1.A	55.70%

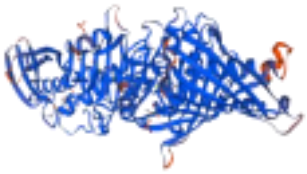
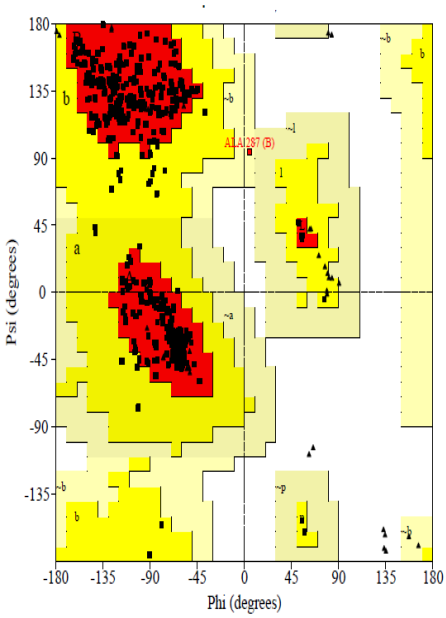
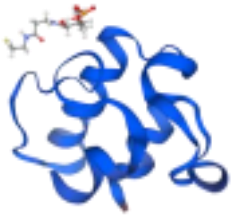
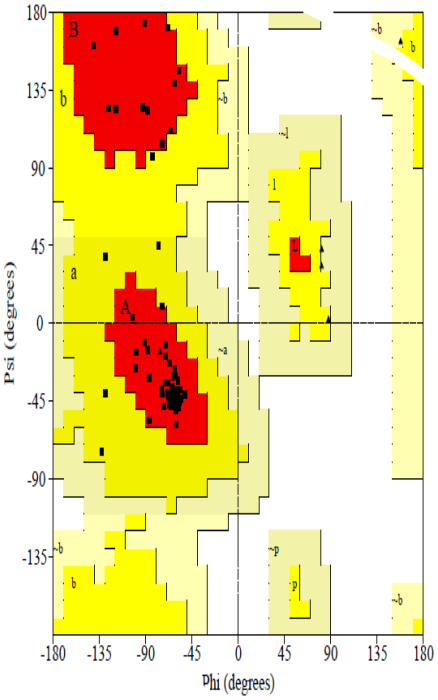
TABLE 2: TABLE OF SEVEN DOMAINS WITH THEIR RESPECTIVE SMTL ID AND SEQUENCE IDENTITY

1.4.7 Structure stability of protein validated by the Ramachandran plot using Procheck server

Protein structures saved in the PDB format from the Swiss model were validated by PROCHECK as shown in the following table 3.

DOMAIN	DOMAIN STRUCTURE	RAMACHANDRAN PLOT
ACP TRANSACYLASE DOMAIN		

BETA-KETOACYL SYNTHASE		
ACYL TRANSFERASE DOMAIN		

PKS PRODUCT TEMPLATE DOMAIN		
PHOSPHOPANTETHEINE ATTACHMENT SITE		

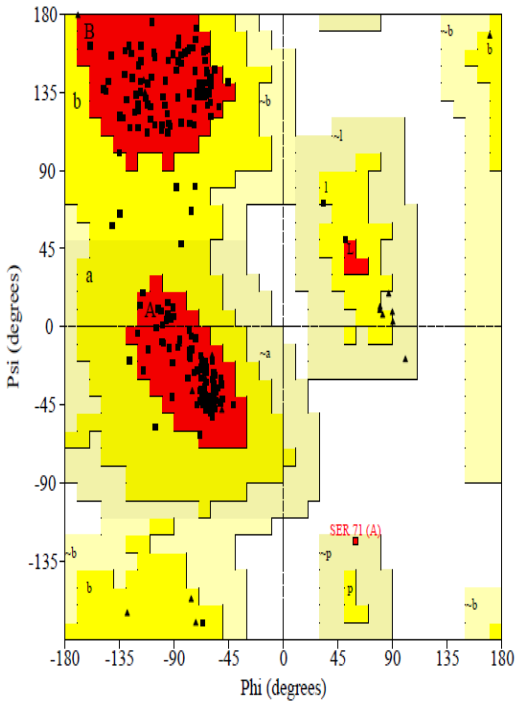
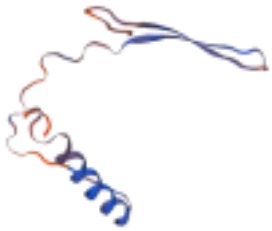
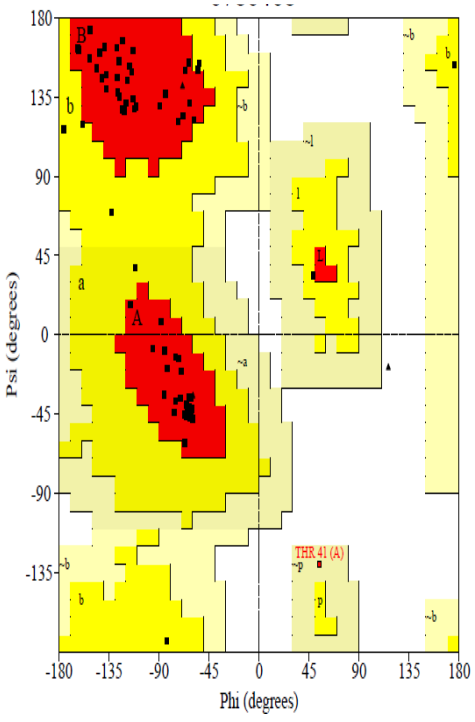
THIOESTERASE DOMAIN		
KETOACYL-SYNTHETASE C TERMINAL EXTENSION		

TABLE 3: STRUCTURE STABILITY VALIDATED BY THE RAMACHANDRAN PLOT USING PROCHECK SOFTWARE

1.4.8 MOLECULAR DOCKING STUDIES

Molecular docking is an intriguing scaffold for comprehending drug biomolecular encounters for rational drug design and discovery including in mechanistic research by placing a molecule (ligand) in a non-covalent way into the selected binding site of the target-specific expanse of the DNA/protein (receptor) to produce a stabilized complex with probable effectiveness and better specificity. The binding energy, free energy, and stability of complexes can all be estimated using the docking technique's data. Currently, the docking methodology is used to estimate the ligand-receptor complex's putative binding characteristics in advance. The basic goal of molecular docking is to create a ligand-receptor combination that has an optimal conformation and has a lower binding free energy. The net anticipated binding free energy (Gbind) is expressed in terms of different characteristics, including hydrogen bond (Ghbond), electrostatic (Gelec), torsional free energy (Gtor), dispersion and repulsion (Gvdw), desolvation (Gdesolv), and total binding free energy (Gbind), total internal energy (ΔGtotal) and unbound system’s energy (ΔGunb). As a result, a thorough grasp of the basic ethics that control anticipated binding free energy (Gbind) yields additional insights into the nature of diverse types of interactions that lead to molecular docking.

Quercetin and Gallic acid were docked with seven distinct domains for A.flavus PksA and compared to the substrate, namely Hexanoic acid, which is known to be involved in PksA activation as well as the control of aflatoxin biosynthesis.

1.4.8.1 DOCKING USING AUTODOCK 4.2

Bioinformatics has finally advanced to the level that it can practically predict the molecular interactions that maintain a protein and ligand-bound in the bound state. The AutoDock software was developed to provide a mechanism for projecting the interaction of micro molecules with macromolecular targets that can readily differentiate amongst compounds with micromolar and nanomolar binding constants, as well as rank molecules with lower affinity differences. Testing a variety of compounds or compounds with specialized binding qualities, alterations to an already existing compound The current work is a full description of the protocol for making AutoDock more user-friendly. The initial step is to locate the Ligand and Target.pdb files that are necessary from major databases. The second step is to use AutoDock 4.2 to create PDBQT format files for Target and Ligand (Target.pdbqt, Lig-and.pdbqt) as well as Grid and Docking Parameter files (a.gpf and a.dpf).

1.4.8.2 Grid Generation (open docking) in AutoDock

1.File -> Read Molecule -> protein.pdb(prepared protein)

2.Edit -> Delete Water

3.Edit -> Hydrogens -> Add H -> Ok

4.Edit -> Charges -> Add Kollman charges

5.Edit -> Charges -> Compute Gastegier charges

6.Atoms -> Assign AD4 type

7.File -> Save -> Write PDBQT

8.Ligand -> Input -> Open -> .mol2 files(prepared ligand) -> Ok

9. Ligand -> Output -> Write PDBQT

10. Grid -> Macromolecule -> Choose -> protein -> Save as .pdbqt

11. Grid -> Grid Box -> adjust the grid covering all of the protein molecules, set the no. of X, Y, Z

dimensions, and spacing accordingly.

12. File -> Close saving current

13. Grids -> Output -> gpf -> save as grid.gpf

• **Auto Grid**

1. Run -> Auto-Grid -> Program Pathname -> Browse -> C Drive -> (XProgram Files) -> The Scripps Research Institute -> Autodock -> 4.2.6 -> autogrid.

2. For parameter filename -> Browse ->grid.gpf.

3. Automatically a log filename file grid.glg will be created -> Launch

• **Auto Dock**

1. Docking -> Macromolecule -> RigidFilename -> protein.pdb

2. Docking -> Ligand -> Choose -> .mol2 file -> Accept

3. Docking -> Output -> Lamarkian -> Save as dock.dpf

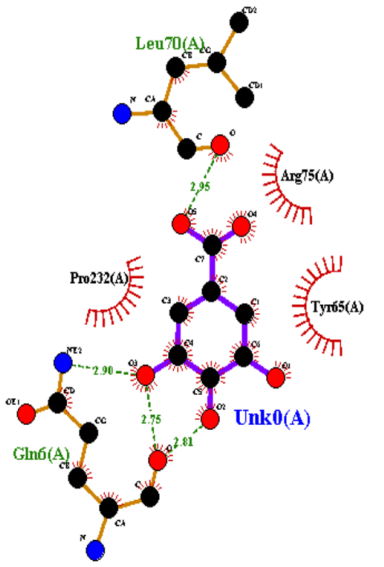
4. Run -> Auto-Dock -> Program Pathname -> Browse -> C Drive -> (XProgram Files) -> TheScripps Research Institute -> Autodock -> 4.2.6 -> autodock.

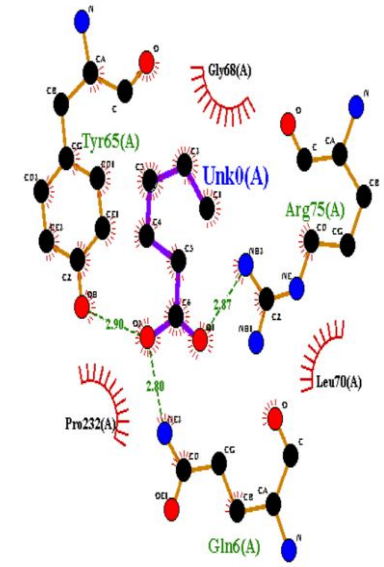
5. For parameter filename -> Browse ->dock.dpf.

6. Automatically a log filename file dock.dlg will be created -> Launch

1.4.9 LIGPLOT ANALYSIS

GALLIC ACID	**HEXANOIC ACID**	**GALLIC ACID**	**HEXANOIC ACID**
ACP TRANSACYLASE DOMAIN		**PT DOMAIN**	





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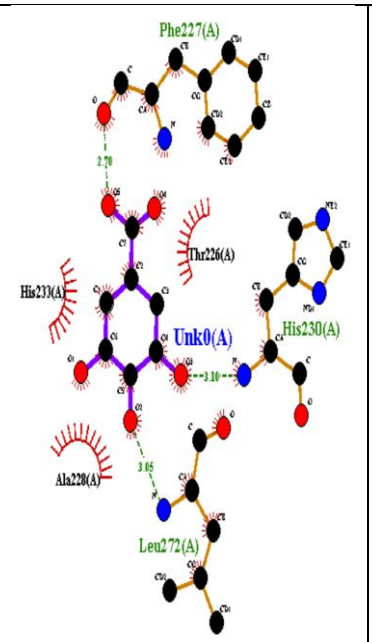
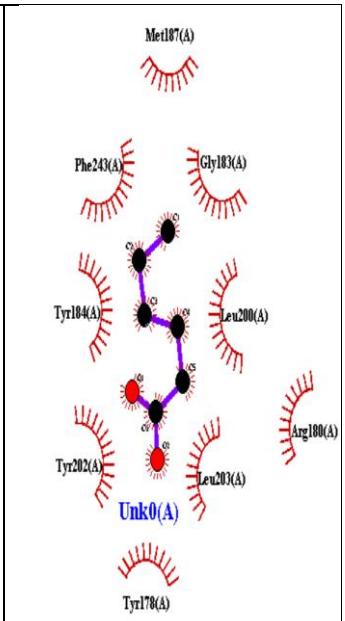
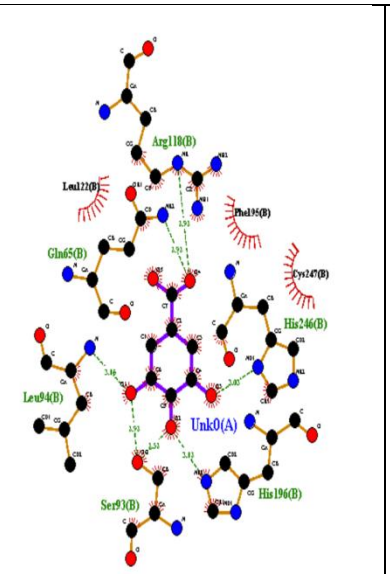
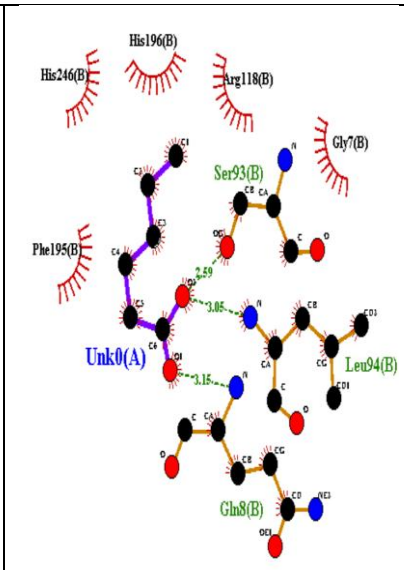


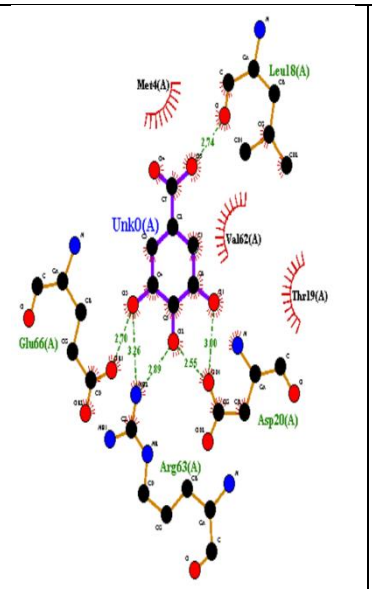


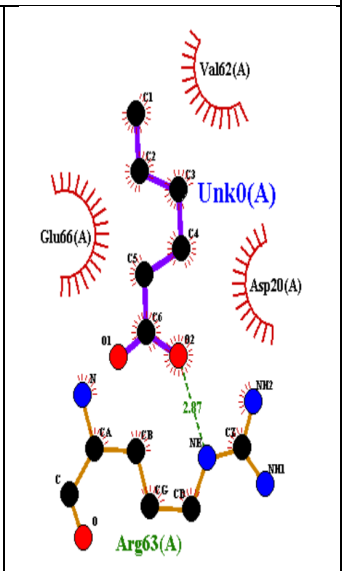
PHOSPHOPANTETHEINE ATTACHMENT SITE





KETOACYL SYNTHETASE C-TERMINAL DOMAIN





B-KETOACYL SYNTHASE DOMAIN

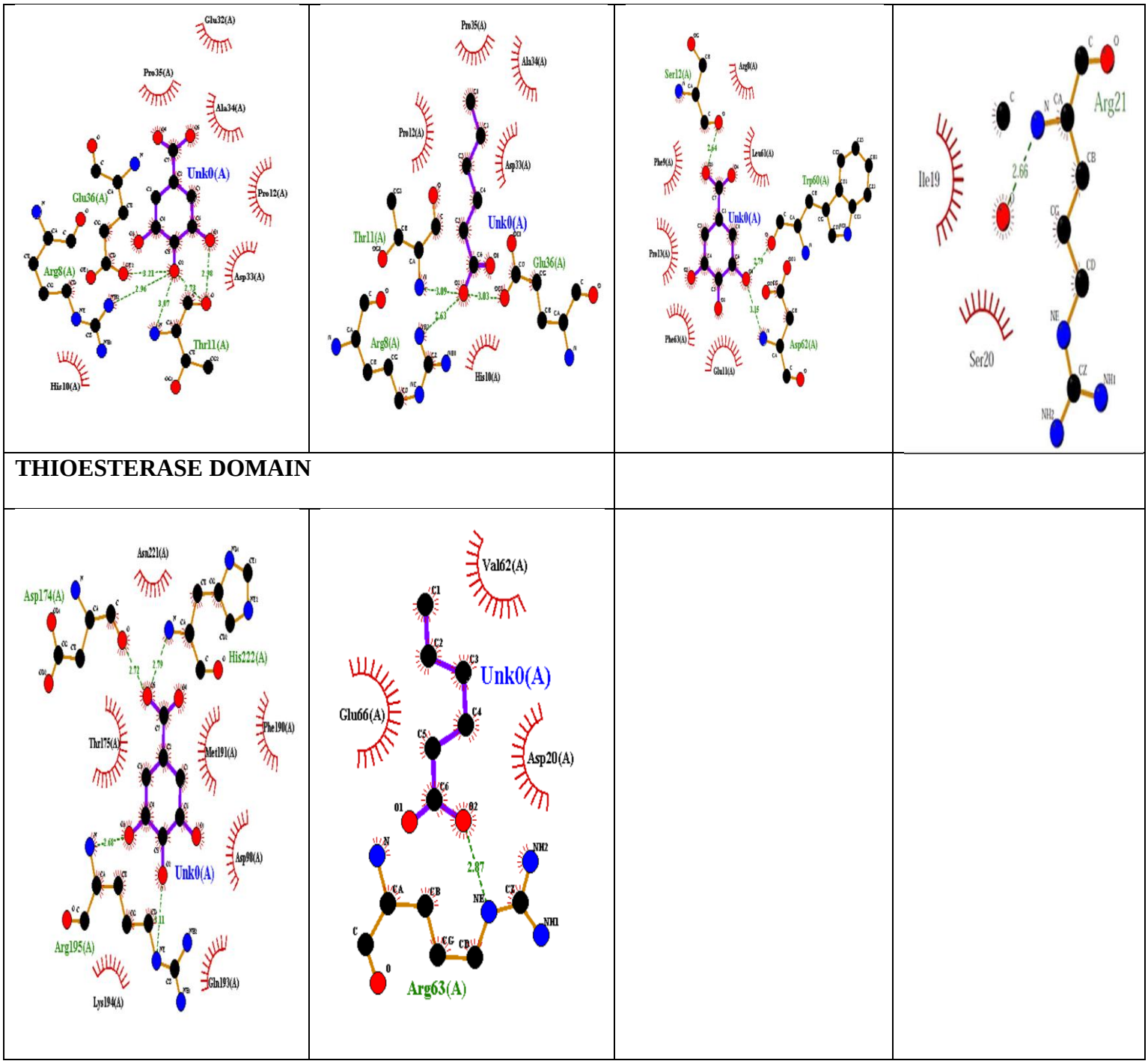


Table 4 - Post-docking correlations among active residues of seven domains of Aspergillus flavus PksA with 2 distinct ligands i.e. Gallic acid and Hexanoic acid, as depicted in a schematic diagram created with LigPlot.

1.4.10 MOLECULAR DYNAMIC SIMULATIONS

MD (molecular dynamics) is a computer modeling technique for studying the physical movements of molecules and atoms. For a fixed period, the atoms and molecules are allowed to interact, providing a glimpse of the dynamic "evolution" of the system. Atomic and molecular trajectories are calculated in the most common version by Using interatomic potentials or force field molecular dynamics, mathematically analyzing Newton's equations of motion for a particle communicating device, whereby forces between both the particles and their potential energies also are gauged.

Simulations are required to see how a molecule attaches to its receptor and how it affects the binding ability of molecules that bind elsewhere (in part by changing the configuration of the protein). Protein-Ligand Complex was prepared using Pymol tool by editing Protein-ligand docked output files, creating the TOP-POSE protein-ligand complex. Parameterization of Protein-ligand Complex was done using Open-Babel and VMD software(http://www.ks.uiuc.edu/Research/vmd/) to start MD simulation by creating topology files for bound ligands. The Solvation Box was created and Force field CHARMm was set as a simulation parameter. MD Simulations were run by creating a system and Minimizing it using NAMD software(https://www.ks.uiuc.edu/Research/namd/) where the results included Analysis of Trajectories, RMSD,RMSF , Hydrogen bonding, and Ramachandran plot which Mimics what atoms do with the potential energy function in real life and analyzes the physical movements of atoms and molecules as provided the positions of the other atoms, the energy equation can be used to measure the force encountered by any atom. Gallic acid simulation analysis is shown in table 5.

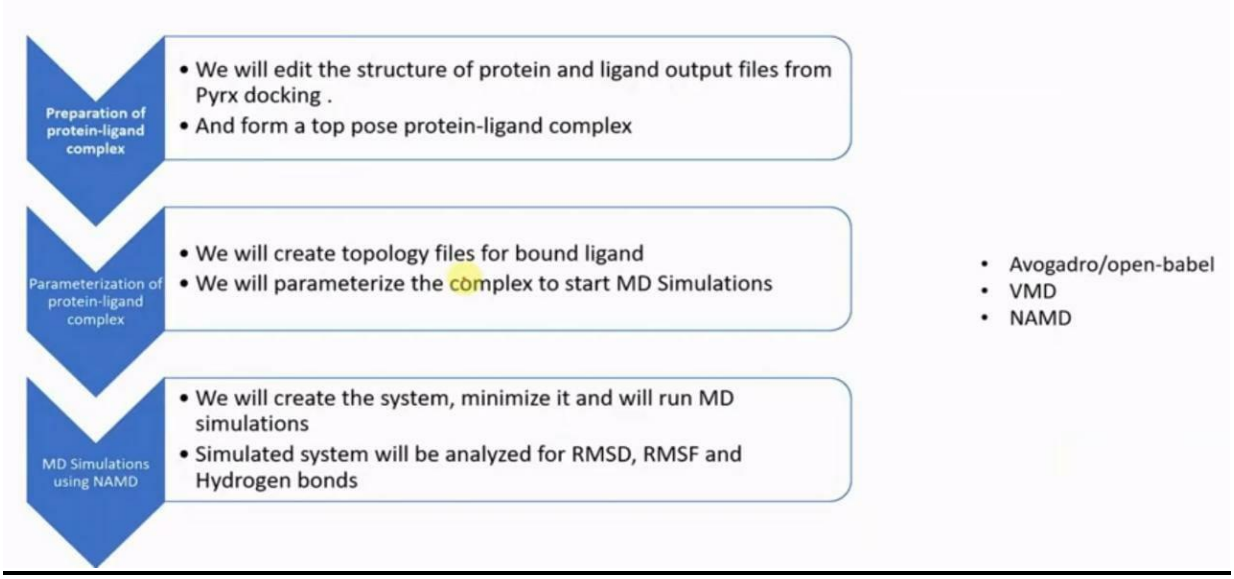


FIGURE 10 : STEPS INVOLVED IN MD SIMULATION

DOMAINS	AVG	STSD	MIN	MAX	NUM
ACP	1.88	0.68	0.09	3.99	102
AT	2.15	0.58	0.09	0.89	102
CT	1.35	0.24	0.04	1.58	102
KS	0.80	0.13	0.05	0.94	102
PP	0.97	0.19	0.04	1.17	102
PT	1.73	0.45	0.05	3.21	102
TH	0.83	0.15	0.03	0.98	102

TABLE 5: TABLE OF SIMULATION OF GALLIC ACID

note - avg, max, min, stdev and num >: When using the -all frames argument, this specifies the rule to use to merge frames. These correspond to retaining the average, maximum, and minimum values from the measured frames' set. The standard deviation for each point over the range of frames will be returned by stdev and num specifies the no. of atoms selected

1.5. RESULTS

1.5.1 HOMOLOGY MODELING, SEQUENCE ALIGNMENT, AND PHYLOGENETIC ANALYSIS

Protein BLAST identified regions of similarity between the biological sequences of proteins by Comparing them with databases of sequences and measuring their statistical importance. The results showed 24 protein sequence (PksA) homologs of more than 90 percentage identity, Based on Query coverage, E-Value and total similarity Ranking, the top 14 homologous sequences have been further analyzed to study the phylogenetic makeup of *A. flavus* PksA. The PksA array of A. Flavus is close to that of *Aspergillus* species, primarily *A. oryzae*, *A. parasiticus*, *A. Pseudo Nomius, A. novoparasiticus*, *A. sergii*, *A. Arachidicola*, and *A. minisclerotigenes*, according to the evolutionary tree shown in Fig. 6. In the UCSF chimera, a three-dimensional structure of both ligands including gallic acid and hexanoic acid was developed, which was visualized in Pymol. Table 1 shows the results of Lipinski's five-rule evaluation.

The results revealed drug-like attributes for these two ligands, along with three - dimensional structure for molecular docking research. The NCBI conserved domain sequence database revealed that *A. flavus* has seven distinct PksA domains. The SWISS-MODEL server has been used to model only those seven PksA domains, and the absolute best model was selected depending upon the sequence specified for homology modeling (TABLE 3). The SWISS-MODEL Template Library uses the BLAST and HHblits scanning engines to find models and match them with target templates. These methodologies apply to experimental methods by ensuring operational alignments at all levels of sequence recognition, indicating the best template mechanism available for our structure. Table 2 shows the sequence of aligned and extracted templates with their respective

template id. By analyzing residue-by-residue geometry and overall structure geometry, the stereo chemical consistency of a protein structure is tested using the Procheck server. The findings showed that 86.7–94.3 percent of all regions were in the most preferred area, 5.3–13.3 percent in the additionally permitted region, 0.0–1.5 percent in the generously allowed region, and 0–1.4 percent in the disallowed region, according to the Ramachandran plot**.** The average goodness factor (G factor) was found to be between -0.02 and -0.24. The ERRAT tool measured the trends of non-bonded interaction of various types of atoms and plotted the magnitude of the error function against the position of the sliding window of 9 residues, which was determined using statistics from highly refined structures. The result indicated the overall quality factors of seven domain structures which ranged between 63.04 - 97.53. As a result, homology models were used in molecular docking studies based on these validations.

1.5.2 DOCKING CORRELATIONS VALIDATED BY LIGPLOT ANALYSIS

Quercetin, Gallic acid was showcased to interact with different A. Flavus domains and was linked to the substrate, Hexanoic acid, which is also active in PksA enhancement and aflatoxin biosynthesis control. **Table 6** shows the prominence-derived dock scores for free binding energy, intermolecular energy, electrostatic energy, and inhibition constant values. In all domains, the binding energy of Gallic acid was observed to be significantly lower than with Hexanoic acid. Gallic acid had binding energy of 6.09 to 4.79 kcal/mol, while Hexanoic acid had binding energy of 5.05 to 3.36 kcal/mol and the electrostatic energy of Gallic acid (0.81 to 0.26 kcal/mol) and Hexanoic acid (0.44 to 0.01 kcal/mol) are highly comparable. Intermolecular energy is directly proportionate to binding energy, which was between 7.58 and 6.29 kcal/mol for Hexanoic acid and

between 6.54 and 4.85 kcal/mol for Hexanoic acid. A reduction in intermolecular energy was observed along with Final Internal Energy which is in the range of -1.68 and -1.03 in Gallic acid and -0.17 and -0.06 in Hexanoic acid, implying that Gallic acid is a more effective binder with these domains. However, Quercetin showed the highest binding energy i.e -6.82 kcal/mol to -4.76 kcal/mol.

The Hydrogen bonding and hydrophobic active sites of Gallic acid, as well as Hexanoic acid with seven main domains of PksA, were identified and compared using LigPlot tools. Out of seven domains, Both Gallic acid and Hexanoic acid showed the maximum inhibition with Thioesterase Domain and lowest inhibition potential with Phosphopantetheine attachment domain. Gallic acid formed 7 H bonds, while Hexanoic acid formed 3 H bonds, according to LigPlot research. When the acyl transferase domain interacts with it, both Gallic acid and Hexanoic acid showed H bond formation at Glu36, Arg8, Thr11 positions. Also, in the Phosphopantetheine attachment domain, Gallic acid creates six hydrogen bonds in contrast to the one in Hexanoic acid. In ACP, TH, KS, PT Domains, No. of H bonds were observed more as compared to Gallic acid and Hexanoic acid. In AT, PP, CT domains, Gallic acid was observed to have more H bonds than Hexanoic acid. Since in correlation to Hexanoic acid, Gallic acid showed the maximum binding long-term potential (maximum proportion of Hydrogen bonding) well with PksA domain; therefore, Gallic acid can inhibit by collegiately binding well to polyketide Synthase domains. This comparative analysis is shown in **table 7**.

PROTEIN DOMAINS	LIGANDS	BINDING ENERGY (kcal/mol)	INHIBITION CONSTANT (uM-micromolar)	INTERMOL-CULAR ENERGY (kcal/mol)	ELECTRO STATIC ENERGY (kcal/mol)	TOTAL INTERNAL ENERGY (kcal/mol)
ACP transacylase domain	**QUERCETIN**	**-6.44**	**19.16**	**-8.23**	**-0.19**	**-2.30**
	GALLIC ACID	**-5.25**	**546.00**	**-6.47**	**-0.27**	**-1.66**
	HEXANOIC ACID	**-4.47**	**531.02**	**-5.96**	**-0.29**	**-0.18**
β-Ketoacyl synthase domain	**QUERCETIN**	**-6.82**	**9.95**	**-8.61**	**-0.49**	**-2.62**
	GALLIC ACID	**-5.29**	**131.53**	**-6.79**	**-0.15**	**-1.23**
	HEXANOIC ACID	**-4.55**	**463.24**	**-6.04**	**-0.40**	**+0.00**
Ketoacyl synthase C-terminal	**QUERCETIN**	**-5.30**	**129.48**	**-7.09**	**-0.13**	**-2.70**

domain						
	GALLIC ACID	**-4.78**	**311.02**	**-6.28**	**-0.32**	**-1.35**
	HEXANOIC ACID	**-3.81**	**1610**	**-5.30**	**-0.37**	**-0.09**
Acyl Transferase domain	**QUERCETIN**	**-4.76**	**324.97**	**-6.55**	**-0.07**	**-2.78**
	GALLIC ACID	**-4.48**	**520.00**	**-5.97**	**-0.10**	**-1.66**
	HEXANOIC ACID	**-3.85**	**1500**	**-5.35**	**-0.19**	**-0.15**
PROTEIN	**LIGANDS**	**BINDING ENERGY**	**INHIBITION CONSTANT**	**INTERMOLECULAR ENERGY**	**ELECTROSTATIC ENERGY**	**INTERNAL ENERGY**
Product template	**QUERCETIN**	**-6.05**	**36.87**	**-7.84**	**-0.17**	**-2.72**

domain						
	GALLIC ACID	**-4.86**	**272.33**	**-6.35**	**-0.23**	**-1.29**
	HEXANOIC ACID	**-3.81**	**1610**	**-5.30**	**-0.37**	**-0.09**
Phosphopantet-heine attachment site	**QUERCETIN**	**-5.21**	**150.85**	**-7.00**	**-0.51**	**-2.72**
	GALLIC ACID	**-4.83**	**287.97**	**-6.32**	**-0.75**	**-1.00**
	HEXANOIC ACID	**-3.36**	**3360**	**-4.85**	**-0.49**	**-0.12**
Thiosterase domain	**QUERCETIN**	**-5.90**	**47.03**	**-7.68**	**-0.15**	**-2.73**
	GALLIC ACID	**-5.97**	**41.79**	**-7.47**	**-0.53**	**-0.88**
	HEXANOIC ACID	**-4.83**	**1470**	**-5.36**	**-0.10**	**-0.15**

TABLE 6 : Comparative analysis of known protein domains and ligands (Gallic acid , Quercetin and Hexanoic acid) on target molecule domains on the basis of Binding energy , Intermolecular energy ,electrostatic energy, Inhibition constant and Total internal energy

PROTEIN DOMAINS	LIGAND	N0. OF HYDROGEN BONDS	HYDROGEN BONDING	BOND LENGTH()	HYDROPHOBIC BONDING
ACP	GA	4	Leu70, Gln6	2.95,2.90,2.81,2.75	Arg75,Tyr65, Pro232
	HA	3	Tyr65, Arg75, Gln6	2.90,2.87,2.80	Gly68,Leu70,Pro232
	QU	5	Asp159 Ser157 Asp162 Cys234	2.90,2.73,2.05,3.06 , 3.08	Cys156,Gly161,Leu147,Arg150, Pro72,Arg160z
CT	GA	5	Glu36, Arg8, Thr11	3.21,3.07,2.98,2.96 ,2.73	Glu32,Ala34,Pro12,Asp33, His10,Pro35
	HA	3	Glu36, Arg8, Thr11	3.09,3.03,2.63	Pro12,Pro35,Ala34,Asp33, CHis10

	QU	3	Glu42, Leu83	2.70,3.15 , 3.08	Thr41 , Ala81 , leu 80 , His49, Arg46 , Ser47 , Asp44 , Lys43
TH	**GA**	4	Asp174, His222, Arg195	3.11,2.68,2.79,2.72	Lys194,Thr175,Asn221,Phe190,Met191,Asp98 ,Gln193
	HA	1	Phe190	3.32	Thr175,Gln193,Val76,Asp98, Met191,Ala173,His222, Ala172,Asp174,Arg195,Lys194
	QU	5	Ala12 Leu21 Leu18 Tyr15	2.68,2.72,3.11,3.02 , 2.63	Phe13 , Ala16 , Val27 , Thr25 , Ala26 , Arg20 , Phe19
PP	**GA**	6	Glu66, Arg63, Leu18, Asp20	2.74,3.00, 2.55, 2.89 3.26, 2.70	Met4,Val62,Thr19
	HA	1	Arg63	2.87	Val62,Asp20,Glu66
	QU	5	Phe68 Ala2	2.82,2.58,2.90,3.29 ,	Leu65 Leu47

			Asp1 Leu45	2.97	Val3
KS	**GA**	3	Ser12, Trp60, Asp62	3.15,2.79,2.64	Arg8, Leu61, Glu11, Phe63, Pro13, Phe9
	HA	2	Ile258	2.89,2.76	Lys242, Leu183, Ile241, Val257 , Trp184, Arg243 Cys180, Ala1
	QU	4	Glu245 Ala1 Cys180	2.97 , 2.93 ,2.94 , 2.99	Arg243 Leu244 Val257 Ile258 Ile241 Leu183 Trp184
PT	**GA**	3	Phe227, His230, Leu272	3.10,3.05,2.70	His233, Ala228 , Thr226
	HA	0	-	-	Met187,Gly183,Leu200,Arg180, Leu203, Tyr178 , Phe243,Tyr184,Tyr202

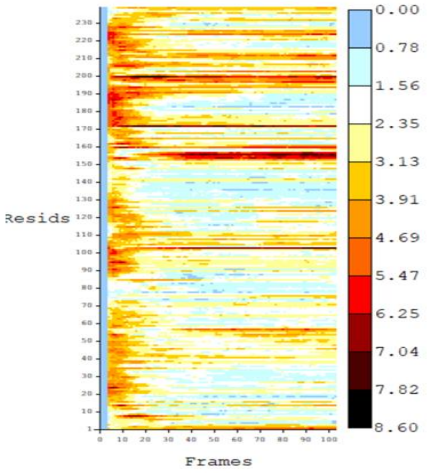
	QU	6	His261, Asn260 Cys45 Tyr50	2.95,2.36,2.96,3.12 , 2.83,2.78	Leu90, Val86, Asp87,Arg315,Ala89, Lys88 Leu44
AT	**GA**	7	His246, His196,Ser93 Leu94,Gln65, Arg118	2.92,2.92,3.03,3.16 , 2.92,2.57,3.12	Leu122,Phe195,Cys247
	HA	3	Gln8,Leu94, Ser93	2.59,3.05,3.15	Phe195,His246,His196,Arg118 ,Gly7
	QU	5	His150,His196,Arg248 , Val154	2.53,2.89,2.79,2.68 , 2.51	Cys152, Glu153,Thr250,His249

TABLE 7 : COMPARATIVE ANALYSIS ON THE BASIS OF HYDROPHOBIC AND HYDROGEN BONDING OF QUERCETIN, GALLIC ACID AND HEXANOIC ACID WITH DIFFERENT PksA DOMAINS OF ASPERGILLUS FLAVUS

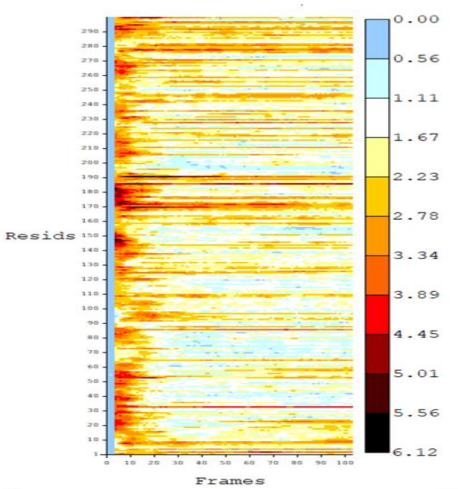
1.5.3 SIMULATION

To gain a better understanding of protein binding affinity and to obtain dynamic insights into complex systems, MD simulations had been conducted . MD simulations were conducted on the processed hits from molecular docking experiments of Gallic acid and Hexanoic acid as reference inhibitor by using VMD as a molecular visualization software that uses 3D graphics and integrated scripting to view, animate, and analyze large bio-molecular structures of protein and ligand and NAMD software has been a parallel molecular dynamics code that uses the CHARMM27 force field as a simulation parameter for elevated simulation of complex bio-molecular systems. Simulations of molecular dynamics are used to model the diverse behavior of molecular systems over time. This system makes it possible for researchers to better comprehend the versatility and complexities of protein-drug binding. To every chosen molecule, a specific simulation system was developed. Open-babel software was used to create simulation parameters for all ligands. Simulations were run in a solvation box with its periodic boundary conditions with systems neutralized by incorporating counter ions to it. To prevent steric clashes, an energy minimization component to every system was performed employing 5000 steps of the steepest descent algorithm and a total force of Thousand kJ/mol. The PME grid size was set 100 x 100 x 100 grid points and the temperature was set as 310 and MD simulations were run for 5000 steps. The root means the square value of Backbone atoms of proteins and energy profiles that can be measured using simulations of both Gallic acid and Hexanoic acid showed that the simulated systems are fairly stable. The RMSD values in the case of Gallic acid were between 0.80 nm and 2.15 nm for pksa - Gallic acid complexes and 0.62 nm and 1.47 nm in pksa-hexanoic acid complexes. Shifting RMSD values within the first few nanoseconds of simulations demonstrate the initial alteration of ligands at the active site of pksa. RMSD measurements were performed for the chosen molecule via the selected frames with a reference point of both specified molecule and its respective frame. The

calculation's results appeared in the results list, where these are plotted in 2D or 3D graphs as shown in Figure 12. The results list contains details about the RMSD calculations that have been completed. Yet more RMSD analyses are made by using the heatmap plot function, selection of an appropriate Heatmap plot configuration. Each RMSD metric could very well resemble a singular data point throughout the heatmap shown in Figure 11. The results of this analysis revealed that each simulation model obtained relatively stable potential energy profiles that remained constant during the simulation cycle. These results suggest that all protein-ligand complex structures are stable and consistent, paving the way for further research into binding modes, important molecular interactions, and free energy calculations. As a result, the RMSD, hydrogen bonding and heat map representation depicted Gallic acid to form more Stable RMSD of protein backbone atoms as well as Significant upside energy profiles in comparison to Hexanoic acid.



GALLIC ACID HEAT MAP



HEXANOIC ACID HEAT MAP

FIGURE 11: HEAT MAP OF GALLIC ACID VS. HEXANOIC ACID

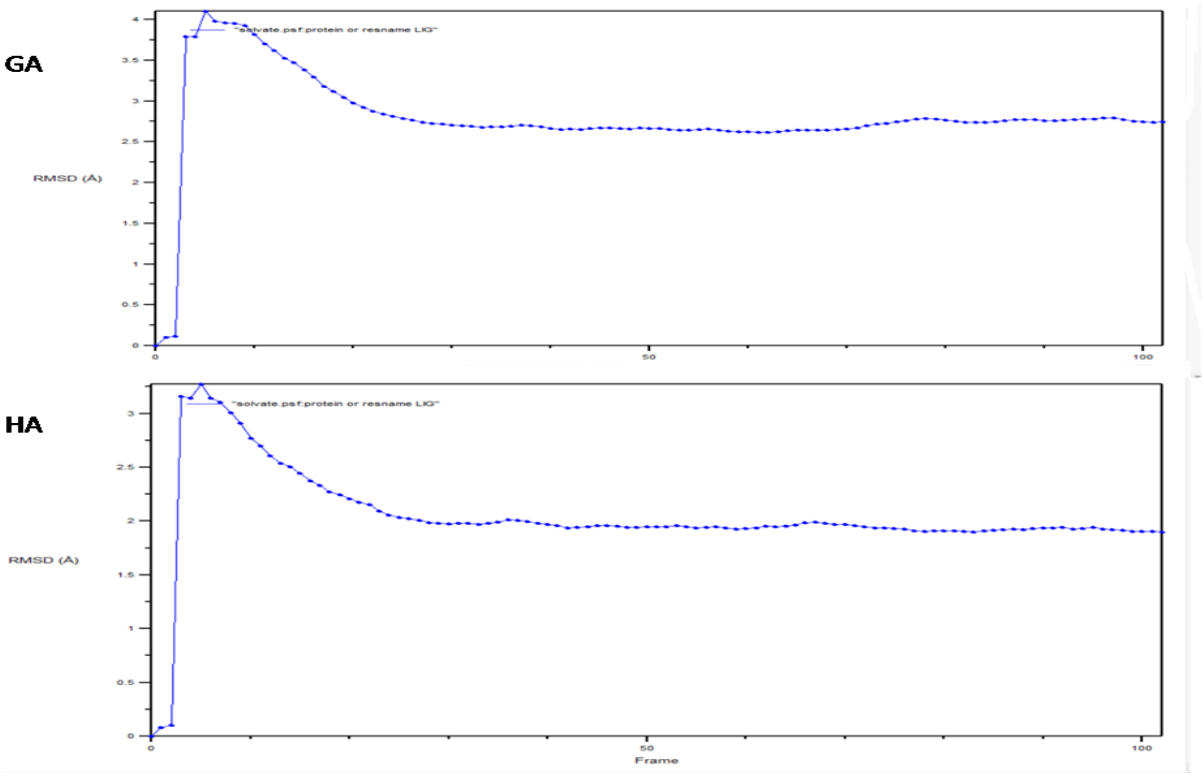


FIGURE 12: RMSD TRAJECTORIES OF GALLIC ACID VS. HEXANOIC ACID

1.6 DISCUSSION

Aspergillus flavus does indeed have an expansive host spectrum as an invasive pathogen which is an unusually prevalent soil fungus. In agriculture, the primary concern with such a fungus is that it induces pharmacologically active toxins called aflatoxin which is carcinogenic in nature and a health threat for animals. In human beings, it is extensively a deceitful pathogen for immunocompromised individuals [5]. Fungal species like *Aspergillus flavus, Aspergillus nomius*, as well as *Aspergillus parasiticus*, afflict plant species throughout development, growing and preservation are responsible for producing these hazardous toxins. These toxins can endure food production and are recognized being an unpreventable food contaminant by the Food and Drug Administration [41]. Synthetically, aflatoxins are derivatives of difuranocoumarin formed via a polyketide pathway. The key four toxins among sixteen structurally analogous toxins are the G1-G2 and B1- B2 aflatoxins [24]. These characters correspond towards both colors underneath ultraviolet light (green or blue) of its fluorescence and to the figures showing their distance measure of mobility on the chromatographic slender plate. The main naturally produced formidable potent is Aflatoxin B1 as a carcinogenic agent [25]. The multi-domain polyketide Synthase A (PksA) enzyme encompasses a hexanoyl starter unit including seven malonyl-CoA adhesive units, which initiates aflatoxin biosynthesis by synthesizing the protonated norsolorinic acid anthrone in the dynamic aflatoxin B1 pathway. Aflatoxins' biosynthesis genes, as well as those of many secondary metabolites, have been grouped [42]. The whole cluster has also undergone sequencing and transcription [37]. The cluster of genes is eighty-two kb long as well as incorporates twenty-five diversified genes. Comprehending aflatoxin biosynthesis is anticipated to boost the creation of control methods that provide an insight into why and how the aflatoxin has been formulated. The

genome of A. Flavus was lately sequenced [43]. The data thereby establish significant tools for comprehension of both fungus and aflatoxin production. Prevention seems to be the ideal solution to limiting the toxicity of aflatoxins Susceptibility to agricultural crop production. Scientists are gradually considering phytochemicals in comparison to conventional compounds to establish approaches to combat fungal diseases globally from the past few years. The term "Phytochemicals" is assigned to naturally produced, non-nutritive bioactive organic compounds of plant sources that have resistant and disease preventive characteristics. Many phytochemicals have become detrimental to fungi but can be utilized to protect crops, livestock, homo sapiens, food, and feed from toxic fungi. And hence, it's indeed vital to examine a feasible, cost efficiency, and non-toxic mechanism for the preventative measures for fungal degradation thereby creating An opportunity to dissuade artificial preservatives implemented by the utilization of natural plant materials and biological control factors. Experimental data demonstrates Gallic acid exemplifies anti-aflatoxigenic and anti-*aspergillus* functions [2]. A contingent of Tulare walnut pellicle, Gallic acid (GA), clearly indicates a significant inhibition effect against aflatoxin biosynthesis [32]. In-vitro analysis on Gallic acid showed significant inhibition against aspergillus flavus PKS A. Domain [44]. As a result, a computationally integrated study with Aspergillus flavus Polyketide Synthase A (PksA) was carried out to understand the mechanisms involved in underlying Phytochemicals suppression of aflatoxin biosynthesis[1].

In our analysis, Gallic acid, as well as Hexanoic acid as a precursor, exhibited similar binding properties by forming a single Hydrogen bond at Phe190 Position and 4 h bond at Asp174, His222, Arg195 positions. It also indicates a greater binding affinity for Gallic acid comparable to Hexanoic acid as the gallic acid’s binding energy was found to be − 6.09 kcal/mol in correlation with Hexanoic acid − 5.05 kcal/mol binding energy in the Thioesterase Domain of PksA . Hydrophobic

interactions indicated that seven amino acids were involved in Gallic acid and eleven amino acids were involved in Hexanoic acid. Photochemical showed stable binding with active site of Polyketide Synthase A (PksA) Demonstrated by persistent RMSD and reliable energy profiles of protein backbone atoms.

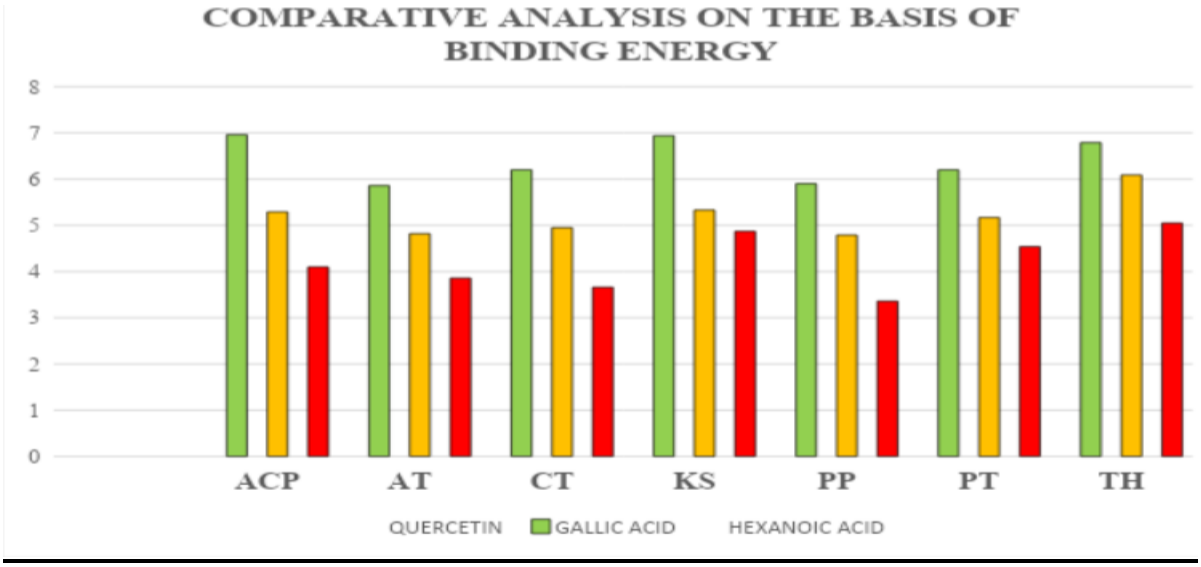
In the interaction with Gallic acid, the amino acids Arg75 and Tyr65 were found in the hydrophobic region although precluded in the interplay of Hexanoic acid with the ACP trans acylase domain as Gly68, Leu70 are involved with one common residue Pro232 in Hydrophobic Bonding. This implies that the ACP transacylase domain of PksA has distinct binding sites for Gallic acid and Hexanoic acid. Gallic acid's binding specificity with the ACP trans acylase domain, on the other hand, was found to be greater than hexanoic acid's in terms of binding energy (5.29 kcal/mol vs. 4.10 kcal/mol)and formation of Four H Bonds in Gallic Acid and Three for Hexanoic acid.In contrast to Hexanoic acid, in-silico techniques revealed active binding of Gallic acid with product template domain. Thus, GA is more stable due to the formation of 3 H bonds at Phe227, His230, and Leu272 positions and no bond formation in Hexanoic acid. In addition, among 7 domains of PksA, both ligands had the lowest electrostatic energy (-0.26/-0.01) when they interacted with the PT domain. Gallic acid showed the Highest Intermolecular energy as compared to Hexanoic Acid (-1.68 /-0.09) with product template domain. In Ketoacyl synthetase C-terminal domain-pksa complex, both GA and HA show hydrogen bonding at Glu36, arg8, thr11 positions but ct-pksa with gallic acid is more stable due to the formation of 5 hydrogen bonds in Gallic Acid as compared to 3 h bonds in Hexanoic acid. Among 7 domains, Acyl transferase domain and Phosphopantetheine attachment site showed the highest no. of hydrogen bonding with Gallic Acid i.e. 7 /6 and 3/1 in Hexanoic Acid Respectively. According to our findings, Gallic acid has a stronger binding affinity in these two domains than Hexanoic acid, since GA needs less binding energy for AT (− 4.82

kcal/mol) as well as PP (− 4.79 kcal/mol) whereas Hexanoic acid required − 3.86kcal/mol and − 3.36 kcal/mol, respectively.

Natural components of polyketide undergo various frameworks and biochemical processes and combine with fatty acid synthesis, a subsection of biosynthetic measures. Thioesterase most frequently performs the penultimate metamorphosis catalyzed by polyketide synthases as well as fatty acid synthases. In silico studies of Gallic Acid's interaction with the thioesterase domain of A. flavus, PksA, revealed that Gallic Acid had greater binding energy than Hexanoic Acid(− 6.09 kcal/mol vs − 5.05 kcal/mol) validated by stronger hydrophobic interactions and 4 h bond formation at Asp174, His222, Arg195 positions in GA as compared to 1h bond at Phe190 position suggesting among 7 domains, As a result, TH plays a key role in inhibiting aflatoxin biosynthesis in A. flavus. Also β-Ketoacyl synthase, The PksA domain demonstrated more extensive binding for Gallic Acid than hexanoic acid in respect of binding ability, including H binding and hydrophobic interactions. The molecular docking and molecular dynamic simulation of both Gallic acid and hexanoic acid with every domain of PksA revealed Gallic acid as a promising target for polyketide synthase inhibition based on in-silico analysis utilizing three - dimensional structure of seven domain category of *A. flavus* and evaluating their properties which include Hydrogen bonding and hydrophobic interactions along with its binding affinity and electrostatic energy. Following is the comparative graphical analysis of three ligands i.e. Quercetin, Gallic acid, and Hexanoic acid where Quercetin then Gallic acid showed the highest inhibition than Hexanoic acid.

COMPARATIVE BINDING ENERGY ANALYSIS

PROTEIN DOMAIN NAME	QUERCETIN (Kcal/MOL)	GALLIC ACID (Kcal/MOL)	HEXANOIC ACID (Kcal/Mol)
ACP	-6.96	-5.29	-4.10
AT	-5.86	-4.82	-3.86
CT	-6.20	-4.95	-3.66
KS	-6.94	-5.33	-4.87
PP	-5.90	-4.79	-3.36
PT	-6.20	-5.17	-4.54
TH	-6.79	-6.09	-5.05



CHAPTER 2 :

Docking Studies on Shikonin as potential Hsp70 and Hsp90 Phytochemical inhibitor of *Aspergillus terreus*

2.1 INTRODUCTION

The fungus *Aspergillus terreus* has emerged as a significant opportunistic pathogen. Cancer rates, viral infections, and organ transplantation have all increased, resulting in secondary fungal illnesses [44]. Mortality, high persistence, lack of early diagnosis, and inherent Amphotericin B (AmB) resistance are all symptoms of *A. terreus. A. terreus* has gotten more attention in recent years due to an increase in immunocompromised patients. It has a built-in resistance to the conventional medication AmB. In *A. terreus*-diagnosed invasive aspergillosis, antifungal therapy options are limited. It is also a big clinical problem these days due to a greater rate (51%) of IA-related mortality. AmB is a broad-spectrum antifungal medication that is widely utilized. AmBRS was found to be very common (98%) all over the world [45].

2.1.1 PHYTOCHEMICALS AS ANTIFUNGAL THERAPEUTIC

Since 2013, the Leading International Fungal Education (LIFE) platform has recorded the burden of severe fungal illnesses in over 5.7 billion individuals around the world. In addition, 1.5 million deaths are estimated each year. As a result, in the current situation, alternative therapeutic procedures are required that are safe, effective, and environmentally friendly. In this case, **Phytochemicals** are the best option for antifungal action because they are natural.

There is a constant quest for new chemicals and their mechanisms of action to develop high-efficacy **antifungals** [19]. Plants are the primary producers of promising natural compounds with

therapeutic potential in the treatment of fungal infections. Identification of novel Phytochemicals, as well as the creation of medications with lower toxicity and greater efficacy, are urgently needed. Anthocyanin, thiols, phenolic, flavonoids, tocopherol, and carotenoids are plant compounds generated from various portions of plants. These have a variety of biological effects, including anti-cancer, antioxidant, antibacterial, and anti-inflammatory characteristics.

2.1.2 SHIKONIN

Shikonin is indeed a naphthoquinone compound that has undergone extensive testing for its pharmacological characteristics including its antioxidant and anti-inflammatory properties as well as its anti-cancerous, antioxidant, and antimicrobial features. Till now, only a few research have been carried on phytochemicals' action mechanism, cytotoxicity, synergy, including their anti-virulence potential. As a result, we have examined some possible Phytochemicals to expand their medicinal applications. We determined that the evaluated Phytochemicals had an inhibitory impact on *A. terreus*, with Shikonin having the highest efficiency of all. Shikonin suppresses *Aspergillus terreus* multiplication by causing an oxidative burst and inhibits the glyoxylate route, which is an energy-recovery process[46].

2.1.3 ABOUT PROTEIN

Shikonin was identified to control the **Hsp70 and Hsp90** proteins of *Aspergillus terreus* in vitro. Shikonin stress is exemplified by these heat shock proteins. Previous research has shown that Hsp70 and Hsp90 blockers can overcome AmBRS in A. terreus and are thought to play a role in antifungal stress response in *Aspergilli*.

Hsp70 protein, which is extremely conserved, has played a key function in the fungal system under diverse stress circumstances. In fungus, the genes producing Hsp70 protein are extremely conserved. Hsp70 is a chaperone-dependent as well as independent protein that is required for fungal growth and development. Hsp70 protein functional differentiation is influenced by the presence of Hsp70 in various cellular regions. In addition, fungal hsp70 was shown to be expressed in humans after diverse infections, suggesting that it may be used as a biomarker for illness[46].

Heat shock proteins (Hsp90 and Hsp70) have recently been identified as potential antifungal targets. They are highly expressed in stressful settings, such as antifungal medication treatments, and have a part in normal biological processes in fungi. Hsp90 and Hsp70 inhibitors have also been found to increase the potency of the drug AmB in *A. terreus*. As a result, a slew of heat shock proteins (Hsp90, Hsp60, and Hsp70) seems to be essential during A. terreus infections. Toxicity has long been a major worry, and medication targets are a new area of research. In the current context, research is focusing on the creation of numerous therapeutic targets to combat numerous fungal-related diseases in both plants and animals. Due to its vast function in fungal survival under stress situations, Hsps is one such important subject to research on. Hsp90 has been presented as an antifungal target in recent years. Against *A. fumigatus* and *Candida albicans*, the Hsp90 inhibitors geldanamycin and its derivatives showed antifungal activity and a synergistic effect with caspofungin. Our research shed light on the biology of *A. terreus*, particularly the lead molecule shikonin. Docking has been done to see if typical medications like Amphotericin B, Shikonin as a phytochemical, and other significant inhibitors have the best inhibitory effect on Aspergillus terreus Hsp70 and Hsp90 proteins [47].

Strain	Agent	MIC values (µg/ml)		FICI	Outcome
		Alone	Combination		
A. terreus (NCCPF860035)	Shikonin	2.30	1.50	0.828	**Additive interaction**
	Amphotericin B	3.86	0.90		

Figure 1: In-vitro combinatorial effect of Shikonin and Amphotericin B in *Aspergillus terreus*

2.2 METHODOLOGY

1. LIGAND PREPARATION of Molecule name: Shikonin and precursors named Geldanamycin and Cantharidin using UCSF Chimera tool.

2. PROTEIN PREPARATION of Targets in *Aspergillus terreus*: Hsp70 (Q0D231) and Hsp90 (Q0CE88) using UCSF Chimera.

3. Retrieval of structures of Standard drug: Amphotericin B from DrugBank.

4. Based on studies done till now, precursors (standard inhibitors of Hsp70 and Hsp90) like Geldanamycin and Cantharidin were selected.

5. Molecular Docking was performed between HSP70 AND HSP90 proteins and Shikonin along with the reference precursors[48].

6. Study of *In silico* Pharmacokinetic properties of Shikonin, standard Drug and other precursor inhibitors with Hsp70 and Hsp90 targets of *Aspergillus terreus*.

2.3 RESULTS

Ligands	**Hsp70 Protein (87.85% identity)**
Shikonin	-3.47
Amphotericin B	-3.10
Cantharidin	-4.61

Ligands	**Hsp90 Protein (81.29% identity)**
Shikonin	-6.70
Amphotericin B	-11.10
Geldanamycin	-8.74

2.4 CONCLUSION

Docking Analysis showed Significant Inhibition of Shikonin as a Phytochemicals and its inhibitory precursors along with comparable results of amphotericin B with Hsp70 and Hsp90 targets of *Aspergillus terreus.*

3.0 CONFERENCE ATTENDED :

NATIONAL CONFERENCE ON BIODIVERSITY AND BIOTECHNOLOGY OF FUNGI & 47TH ANNUAL MEETING OF MYCOLOGICAL SOCIETY OF INDIA

DATE: February 22-24, 2021.

3.1 POSTER PRESENTED :

DOCKING ANALYSIS OF GALLIC ACID AND HEXANOIC ACID WITH POLYKETIDE SYNTHASE, A COMPUTATIONAL APPROACH TO CONTEMPLATE GALLIC ACID - MEDIATED AFLATOXIN BIOSYNTHESIS INHIBITION IN *ASPERGILLUS FLAVUS*

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BACKGROUND

Aspergillus flavus is the major opportunistic contaminant known to produce aflatoxin in food crops. Food contamination via aflatoxins is indeed a nutriment safety concern for agricultural yields. In Human beings, it is extensively deceitful pathogen for immunocompromised individuals. In order to identify and develop novel anti-aflatoxigenic agents, explorative Studies on phytochemicals as anti-aflatoxigenic agents have accrued remarkable importance including Gallic acid which plays a significant role in providing defense mechanism against it. Thus, to understand the molecular mechanism behind inhibition of aflatoxin biosynthesis by Gallic acid and Hexanoic Acid as a precursor , an interactive study with Polyketide Synthase A (PksA) of *Aspergillus flavus* was à fond conducted.

OBJECTIVES

TO STUDY PHYTOCHEMICALS AS ANTI-AFLATOXIGENIC AGENTS VIA COMPUTATIONAL APPROACH TO UNDERSTAND THE MOLECULAR MECHANISM BEHIND INHIBITION OF AFLATOXIN BIOSYNTHESIS BY GALLIC ACID AND HEXANOIC ACID AND THEIR INTERACTIONS WITH POLYKETIDE SYNTHASE PksA OF *ASPERGILLUS FLAVUS.*

METHODOLOGY

Biological Data Collection And Sequence Retrieval(UniProt ID :B8NI04_ASPFN) followed by Homology Modeling, pBlast analysis associated with Phylogenetic Codification.Ligand preparation of Gallic acid and Hexanoic acid and Analysis of their molecular properties using Lipinski's rule of five and Protein preparation and Structure stability of protein validated by the Ramachandran plot using procheck server was performed.Gallic acid has been docked with the different PksA domains of A. Flavus and, compared to the substrate, viz. Hexanoic acid , which participates in the PksA activation and control of aflatoxin biosynthesis .Ligplot analysis was used to compare the site of H bonding and hydrophobic interaction of Gallic acid and Hexanoic acid with seven distinct domains of PksA.The dynamics of protein-ligand complex formation for every domain was investigated through MD simulations.

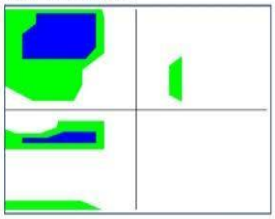
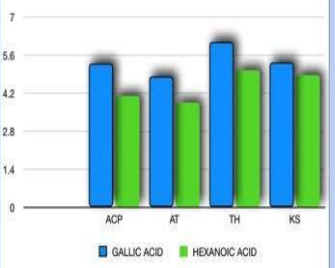


FIGURE 1 :Ramachandran plot analysis indicated most of the protein residues are in the favorable regions

RESULTS & DISCUSSION

Significant Derived Dock scores such as binding free energy, intermolecular energy, electrostatic energy and inhibition constant value results revealed that the binding energy of Gallic acid was found to be less than that of Hexanoic acid in all the respective domains. The binding energy of Gallic acid ranged between − 6.09 and − 4.79 kcal/mol and that of Hexanoic acid between − 5.05 and − 3.36 kcal/mol.



Comparative Analysis on the Basis of Binding Energy

Out of seven domains, Both Gallic acid and Hexanoic acid showed the maximum inhibition with Thioesterase Domain .LigPlot analysis showed the formation of 7 H bonds in Gallic acid and 3 H bonds in Hexanoic acid. Phytochemicals showed stable binding with active site of Polyketide Synthase A (PksA) indicated by steady RMSD of protein backbone atoms and potential energy profiles.

CONCLUSIONS

Our results indicated that Gallic acid exhibited the highest level of binding potential with PksA domain named Thioesterase domain in comparison to Hexanoic acid; thus, Gallic acid feasibly inhibits by virtue of competitively binding to the seven domains of Polyketide Synthase, a critical enzyme of aflatoxin biosynthetic pathway. our study may find its application in Phytochemicals based anti-aflatoxigenic agents .

ACKNOWLEDGEMENT

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