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Virtual screening of siddha formulation *Shaya chooranam* towards identification of potential inhibitor targeting Enoyl-Acyl carrier protein reductase (Inha) in mycobacterium tuberculosis

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Abstract
The worldwide spreading of multidrug resistant Mycobacterium tuberculosis (Mtb) has incited an earnest need to discover novel anti-tuberculous agents. Enoyl-acyl carrier protein reductase (InhA) from Mycobacterium tuberculosis is a well-known and thoroughly studied target for anti-tuberculosis therapy. Herbs have assumed a noteworthy role in the development of several clinically useful therapeutic agents since time immemorial. The point of the current research was to distinguish the lead compounds from Siddha herbal formulation *Shaya chooranam* to find the binding mechanism with the suitable targets against Mycobacterium tuberculosis. Bioactive compounds for the phytocompounds of the ingredients of *Shaya chooranam* such as β-asarone, Quercetin, Gallic acid, Myristic acid, Eugenol and Guineensine were retrieved from different database and docking calculations were performed using Ethambutol as standard. Molecular docking was performed using Autodock 4.2 to analyse the binding affinity with the target protein Enoyl-acyl carrier protein reductase (InhA) and docked compounds were visualized using pymol version 2.7.The results showed that among the phytocompounds quercetin showed the highest hydrogen bond interaction and thus the potential compound against InhA sharing the highest similarity with the standard drug Ethambutol (-4.41Kcal/mol) and further studies are warranted to prove its efficacy

Keywords: molecular docking, *shaya chooranam*, tuberculosis, herbal medicine, siddha

Introduction
Tuberculosis (TB) is one of the most old maladies of humankind and has co-advanced with people for a huge number of years or maybe for a few million years.[1] According to WHO, TB is an overall pandemic. Among the 15 nations with the most elevated assessed TB rate rates, 13 are in Africa, while half of every single new case are in six Asian nations, viz., Bangladesh, China, India, Indonesia, Pakistan and Philippines. A WHO fact sheet dated March 2010 on tuberculosis expressed that general 33% of the total populace (more than 2 billion) is as of now contaminated with the TB bacillus. In India, TB has been referenced in the Vedas and the old Ayurvedic scriptures [2-3]. The antiquated Siddha writings coins the term *Sayam*, *Shayam*, *Ilaippu Noi* and different other *Tamil* and *Sansrkrit* terminologies for Tuberculosis. In this aspect the present study identified lead compounds from Siddha classical formulation *Shaya chooranam* which has been indicated for the management of *Shayam* (Tuberculosis) in the ancient Siddha literature *Agathiyar Koumathi Nool – 400* [4]. Shaya Chooranam includes five herbal ingredients such as *Thippili* (*Piper longum*), *Kadukkai* (*Terminalia chebula*), *Saadhikkai* (*Myristica officinalis*), *Sitrarathai* (*Alpinia galanga*) and *Vaal milagu* (*Cubeba officinalis*) [4].
Medicinal plants play an important role in therapeutics and are the reservoirs of phytochemicals for the management of human ailments. They comprise an effective source of both traditional and modern medicines. Phytochemicals are naturally stored in the different parts of plants such as leaves, flowers, vegetables, root, stem, and bark. These constituents have definite physiological action on the human body [5]. The latest computer techniques i.e. docking studies for the selection of specific phytochemicals on various protein targets have been extensively used in recent days. It also includes literature search to identify some important natural compounds that can be used as analogues and its derivatives can be synthesized to get effective targets [6]. In recent years, many natural herbal formulations have been explored and the benefits derived from herbal constituents for tuberculosis have been proved very promising. Hence the main aim of the present study is to derive the lead compounds β-asarone, Quercetin, Gallic acid, Myristic acid, Eugenol and Guineensine from the ingredients of *Shaya chooranam* and to perform molecular docking using computational analysis to find the binding affinity of the lead compounds against the target protein Enoyl-acyl carrier protein reductase (InhA) of Mycobacterium tuberculosis.

Materials and Methods
Selection and Preparation of target
The targets protein the Enoyl acyl carrier protein reductase (InhA) aids in the formation of type II fatty acid biosynthesis pathway of *M. tuberculosis*. The InhA may act as a potential therapeutic agent for management of Mycobacterium tuberculosis infection. Crystalline structure of the target protein Enoyl acyl carrier protein reductase (InhA) (2NSD) AS shown in Fig. 1 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added.

Preparation of Ligands

The phytocompounds from the major herbal ingredients *Piper cubeba, Alpinia galangal, Terminalia chebula, Myristica fragrans* and *Piper longum* of *Shaya Chooranam* were retrieved from different database and prepared docking by adding gasteiger partial charges added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined detailed in Fig.2.

Docking Analysis

Docking calculations were performed for the phytocompounds β-asarone, Quercetin, Gallic acid, Myristic acid, Eugenol and Guineensine. The drug Ethambutol was used as standard. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added according to Morris, Goodsell et al., 1998. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program [7]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking with a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å.

Results

The phytocompounds of *Shaya chooranam* namely β-asarone, Quercetin, Gallic acid, Myristic acid, Eugenol and Guineensine showed varied level of binding affinity which was compared with the standard drug Ethambutol. The entire summary of the molecular docking studies of the phytocompounds and standard drug Ethambutol against 2NSD is tabulated in detail. The physiochemical properties, Interaction profile of ligand, Amino acid Residue Interaction of Lead and Standard and ranking of compounds have been detailed in Tables 1-3 and Figures 2a -2f.

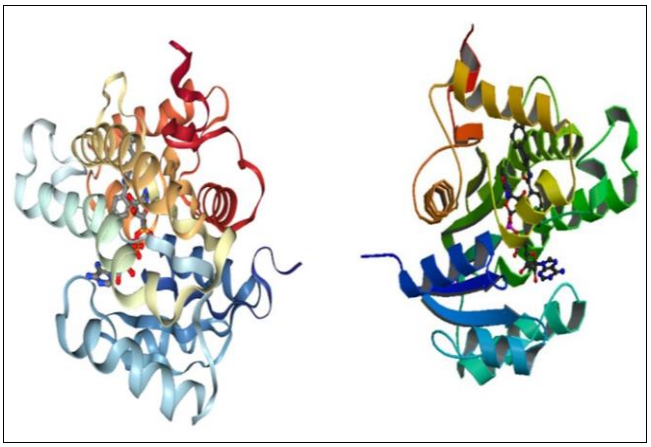
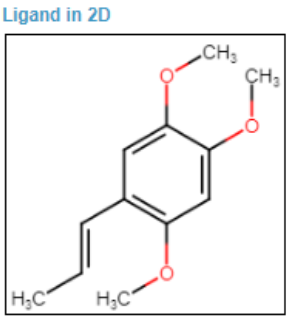
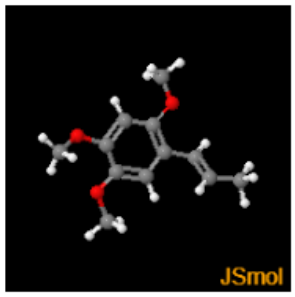
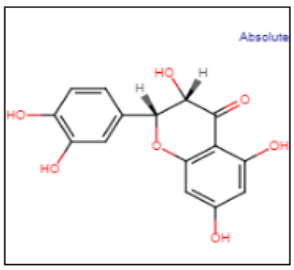
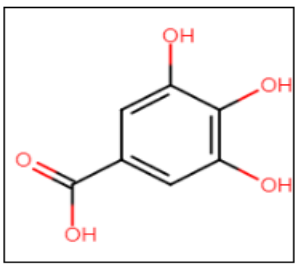
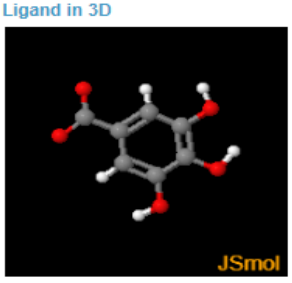


Fig 1: Enoyl acyl carrier protein reductase (InhA) -2NSD

Ligands	2D structure of Ligands	3D structure of Ligands
β-asarone		
Quercetin		
Gallic acid		

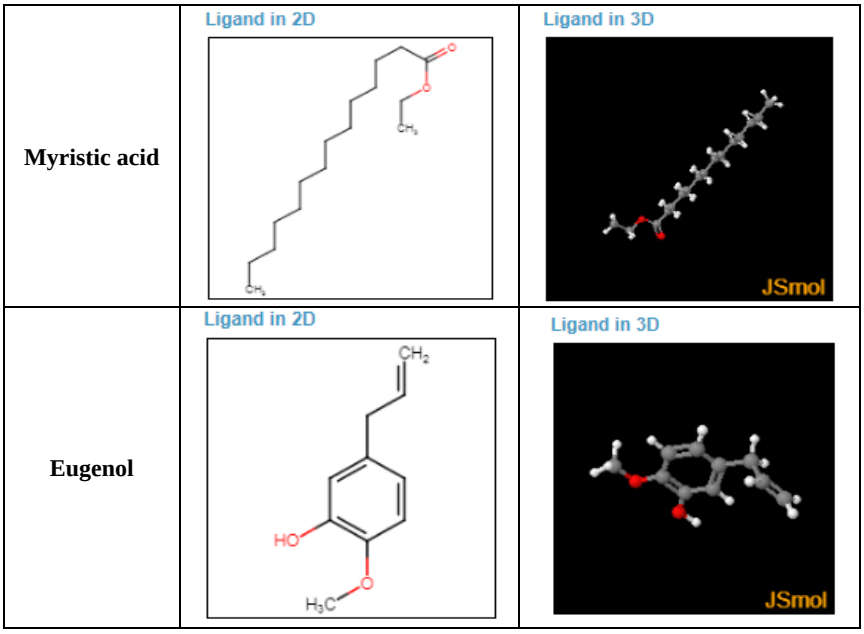


Fig 2: 2D and 3D structure of ligands

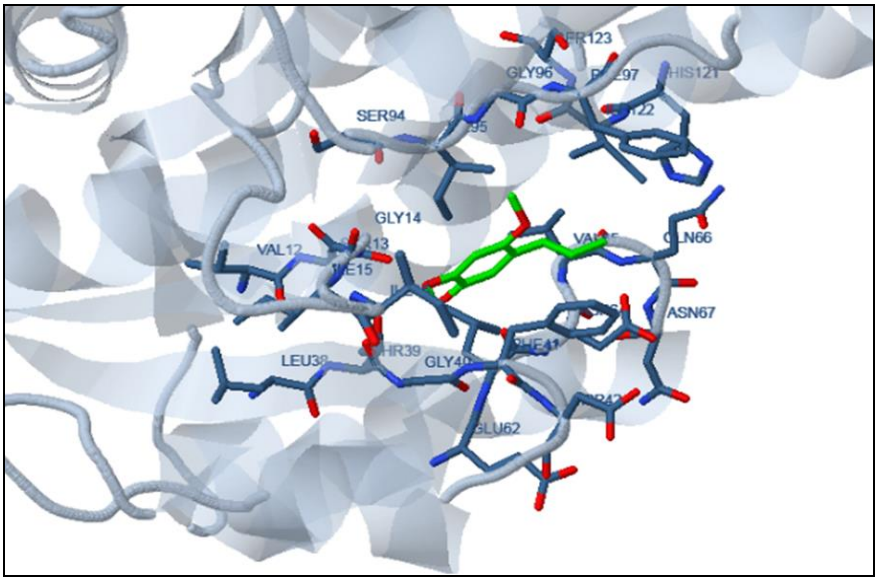


Fig 3: β-asarone with Enoyl acyl carrier protein reductase (InhA) -2NSD

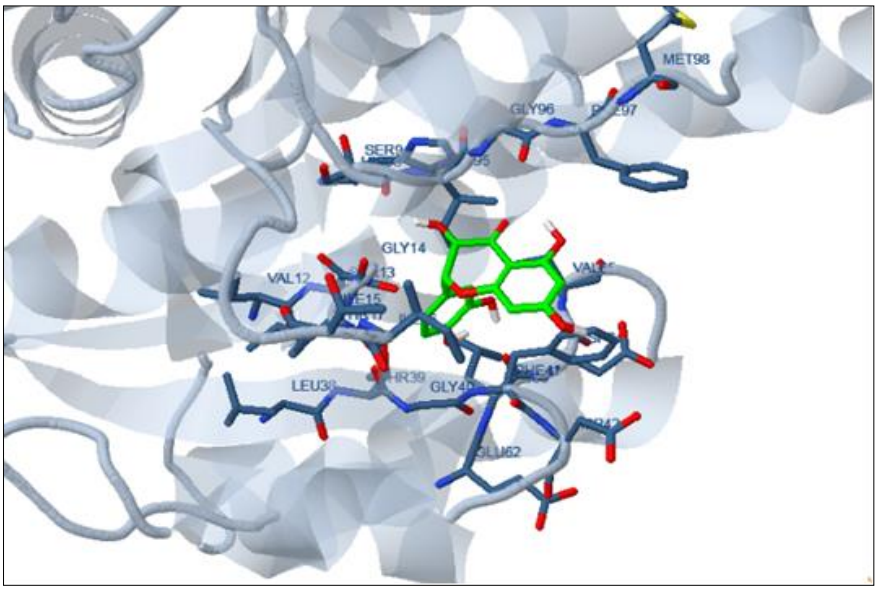


Fig 4: Quercetin with Enoyl acyl carrier protein reductase (InhA) -2NSD

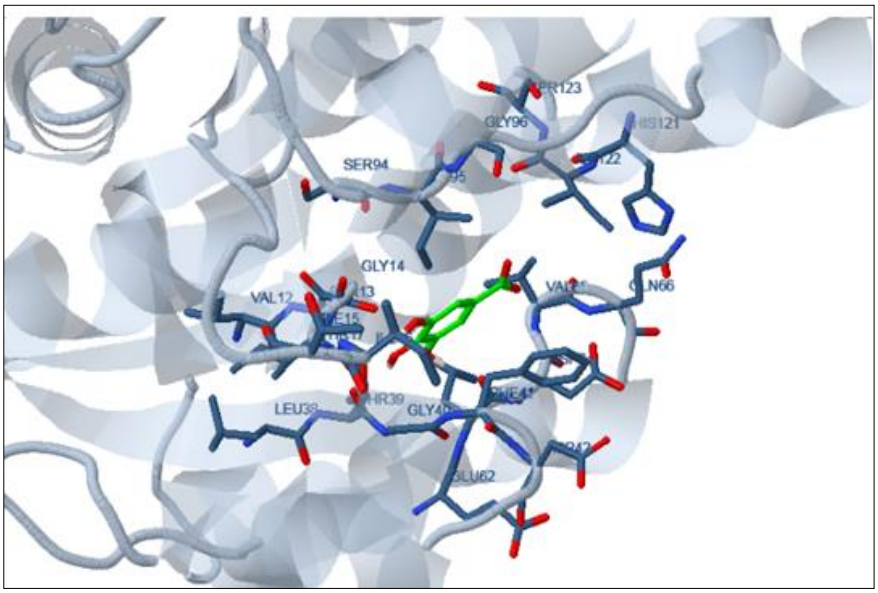


Fig 5: Gallic acid with Enoyl acyl carrier protein reductase (InhA) -2NSD

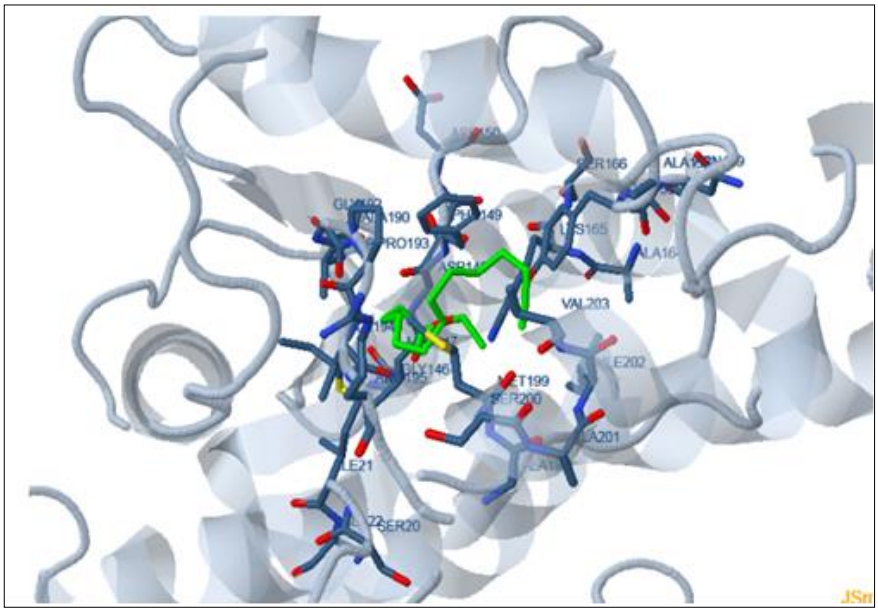


Fig 6: Myristic acid with Enoyl acyl carrier protein reductase (InhA) -2NSD

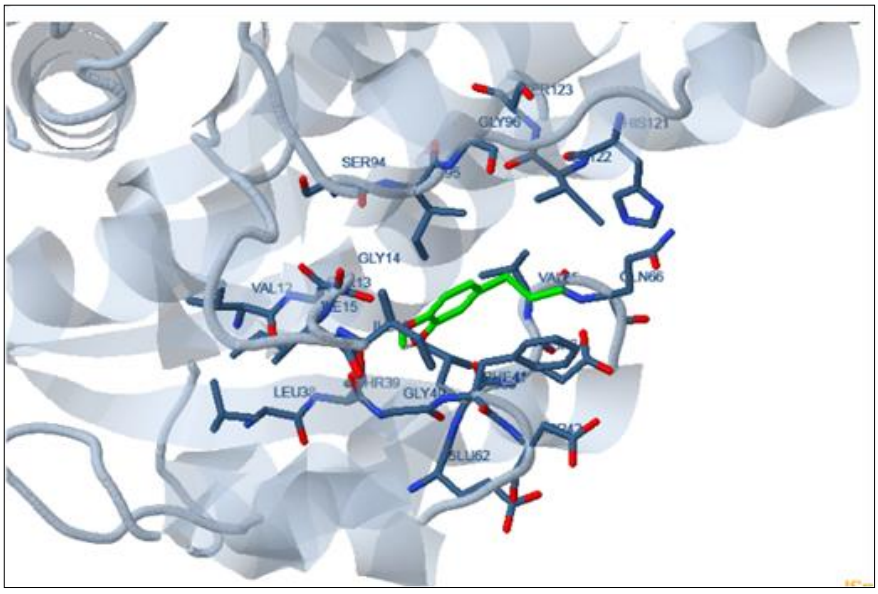


Fig 7: Eugenol with Enoyl acyl carrier protein reductase (InhA) -2NSD

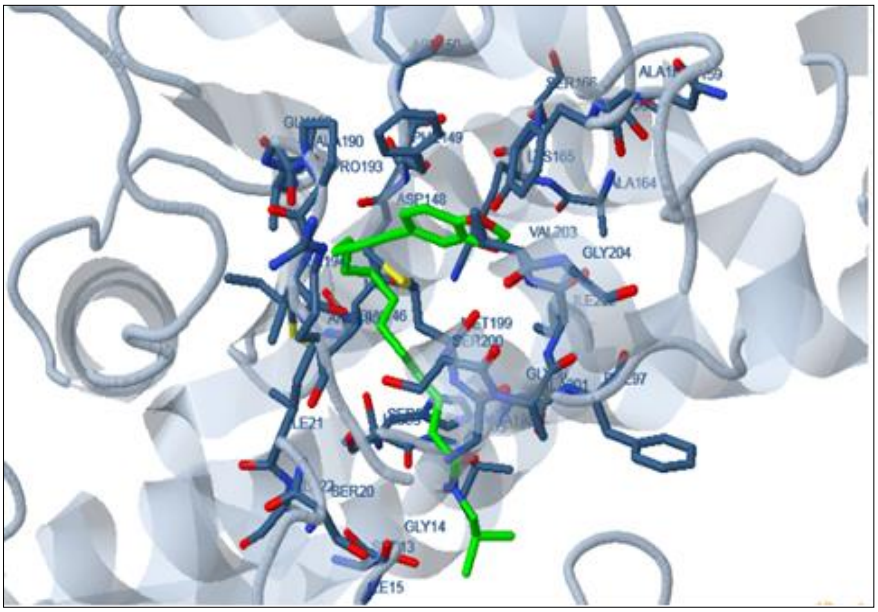


Fig 8: Guineensine with Enoyl acyl carrier protein reductase (InhA) -2NSD

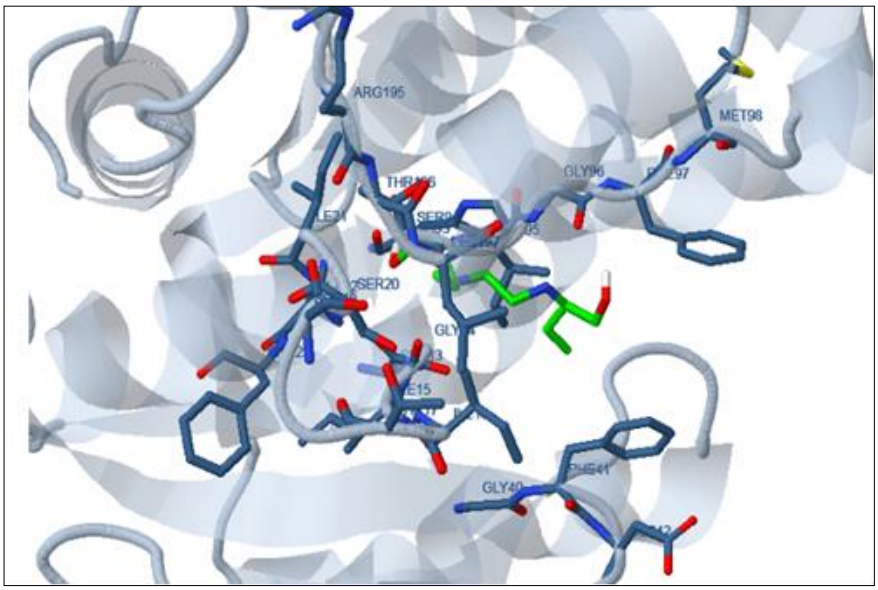


Fig 9: Ethambutol with Enoyl acyl carrier protein reductase (InhA) -2NSD

Table 1: Physiochemical properties of Ligand molecules

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
β-asarone	208.25 g/mol	C12H16O3	0	3	4
Quercetin	302.23 g/mol	C15H10O7	5	7	1
Gallic acid	170.12 g/mol	C7H6O5	4	5	1
Myristic acid	228.37 g/mol	C14H28O2	1	2	12
Eugenol	164.2 g/mol	C10H12O2	1	2	3
Guineensine	383.5 g/mol	C24H33NO3	1	3	12
Ethambutol	204.31 g/mol	C10H24N2O2	4	4	9

Table 2: Interaction profile of ligand against- Enoyl acyl carrier protein reductase (InhA) – PDB 2NSD- Mycobacterium

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
β-asarone	-5.73	62.76	0.01	-6.97	572.5
Quercetin	-5.21	151.48	-0.20	-7.54	648.14
Gallic acid	-5.02	208.35	-0.04	-5.29	424.51
Myristic acid	-5.62	75.88	-0.16	-9.05	735.60
Eugenol	-5.38	113.2	0.02	-6.00	481.99
Guineensine	-8.78	365.5	-11.92	- 11.9	1149.84
Ethambutol	-4.41	582.6	-0.48	-7.31	599.07

Table 3: Amino acid Residue Interaction of Lead and Standard against Enoyl acyl carrier protein reductase (InhA) – PDB 2NSD-Mycobacterium

Name of Phytocomponents	Number of Interactions	Amino Acid Residue- Binding									
β-asarone	2	13 SER	39 THR	41 PHE	63 LEU	64 ASP	65 VAL	66 GLN	95 ILE	122 ILE	
Quercetin	4	13 SER	16 ILE	39 THR	41 PHE	63 LEU	95 ILE	97 PHE			
Gallic acid	2	13 SER	39 THR	41 PHE	63 LEU	65 VAL	65 VAL	95 ILE	122 ILE		
Myristic acid	0	21 ILE	147 MET	149 PHE	158 TYR	165 LYS	191 ALA	193 PRO	194 ILE	199 MET	202 ILE
Eugenol	2	13 SER	39 THR	41 PHE	64 ASP	65 VAL	95 ILE	122 ILE			
Guineensine	2	21 ILE	94 SER	95 ILE	147 MET	149 PHE	158 TYR	165 LYS	191 ALA	199 MET	202 ILE
Ethambutol	9	14 GLY	16 ILE	20 SER	22 ALA	41 PHE	94 SER	95 ILE	96 GLY	97 PHE	

[a] SER- Serine [b] THR-Threonine [c] PHE -Phenyl alanine [d] LEU-Leucine [e] ASP-Aspartate [f] VAL-Valine [g] GLN- Gluatamine [h] ILE-Isoleucine [I] MET-Methionine [j] TYR-Tyrosine [k] LYS-Lysine [l] ALA-Alanine [m] PRO-Proline

Table 4: Ranking of compounds based on Amino acid residue interactions on Enoyl acyl carrier protein reductase (InhA) – PDB 2NSD- Mycobacterium

Name of Phytocomponents	Number of Interactions	Rank
β-asarone	2	II
Quercetin	4	I
Gallic acid	2	II
Eugenol	2	II
Guineensine	2	II

[a] Ranking based on binding energy and hydrogen bond interactions

Discussion

Tuberculosis (TB) is a fatal infectious disease caused by *Mycobacterium tuberculosis*, infecting one-third of the world's population [9]. Despite of the various drugs available in the market to treat tuberculosis there is still need for an effective drug due to the increasing resistant strains emerging frequently [10]. In this study we have performed a docking study on the protein Enoyl acyl carrier protein reductase (InhA) which is responsible for type II fatty acid biosynthesis pathway of M. tuberculosis. The protein used in the study is the InhA enzyme with the PDB id 2NSD. The Isoniazid is believed to be a potential target of the enzyme. Isoniazids interfere with NAD-utilizing enzymes, primarily the enoyl-ACP reductase thus arresting the mycolic acid synthesis essential for *M* [11-12]. Hence the present study targeted the enzyme InhA, where the potential phytocompounds of the medicinal formulation *Shaya chooranam* such as *Piper cubeba, Alpinia galangal Terminalia chebula, Myristica fragrans, Piper longum* have been screened for lead compounds. The selection of this formulation *Shaya chooranam* is based on the available Sidddha literature evidences for the management of tuberculosis in Siddha medicine. The phytcompounds were evaluated for the Lipinski rule violation and it was observed that all the phytocompounds satisfied the rule.

Lead compounds

Guineensine

Guineensine has a benzodioxane moiety terminal to the alkyl chain. It belongs to a class of unique natural Nisobutylamides and was first isolated from West African pepper (Piper guineense Schumach). This fatty acid derived natural product is abundant in several species of the plant genus Piper, including the dietary pepper species P. longum L. and P. nigrum L [13-14].

Gallic acid

Gallic acid is a colorless or slightly yellow crystalline natural phenolic compound called 3, 4, 5-trihydroxybenzoic acid found in several fruits and medicinal plants. It is reported to have several health-promoting effects and abundantly present in the plant kingdom. Gallic acid has been isolated from different plant species such as *Quercus* spp. And *Punica* spp., via various chromatographical methods. Previous studies indicate that Gallic acid can inhibit HIV-1 integrase, HIV-1 transcriptase, HIV-1 protease dimerization, HCV attachment and penetration, HCV replication, HCV serine protease, the herpes simplex virus (HSV)-1 and HSV-2 attachment and penetration. It also causes disruption in *Haemophilus influenza* A and B particles. [15]

β-asarone

β-asarone (2, 4, 5-trimethoxy-(*Z*)-1-propenylbenzene) was the main constituent (70 to 90%) of rhizomes of herbs[16] It possesses anti-tumor and chemo-preventive activities as evident from numerous pre-clinical studies both *in-vitro* and *in-vivo*. The study done by Qi *et al* in conclusion, suggested that (β)-asarone inhibits invasion and EMT in human glioma U251 cells by suppressing splicing factor hnRNP A2/B1 [17].

Myristic acid

Myristicin, constitute about 80% of the alkenylbenzene derivatives of Myristica fragrans (Nut meg) [18]. Esters and amides of Myristic acid was synthesized and tested in vitro for antibacterial activity against gram-positive and gram-negative bacteria. All the compounds showed activity comparable to that of the standard drug, ciprofloxacin. [19]

Eugenol

Eugenol (4-allyl-2-methoxyphenol (EUG)) is a terpene and is an essential oil from the Piper species [20]. It presents itself as yellow viscous oil at normal temperature. Previous studies have reported biological activities of Eugenol as antibacterial, antifungal and antiallergic agent. With regard to the inflammatory process, several studies demonstrate the anti-inflammatory activity of EUG. It was shown that Eugenol promoted a reduction in carrageenan-induced pleural volumes in mice. Furthermore, Eugenol causes inhibition of proinflammatory mediators such as COX-2, NF-κB, IL-6, leukotriene C4, and 5-LOX [21].

Quercetin

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is yellow colour phytochemical found in Terminalia Chebula [22]. The name quercetin comes from the Latin word "Quercetum" which means Oak Forest, belongs to the class called flavonols that cannot be produced in the human body. It is poorly soluble in hot water, quite soluble in alcohol and

lipids and is insoluble in cold water. Quercetin is said to be one of the most widely used bioflavonoids for the treatment of metabolic and inflammatory disorders. This potent plant pigment is an antioxidant and more specifically a bioflavonoid present in more than twenty plants material and known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities [23]. Quercetin, a plant-derived aglycone form of flavonoid glycosides, has been used as a nutritional supplement and may be beneficial against a variety of diseases including cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, and anti-infective action [24].

Binding affinity of lead compounds with Enoyl acyl carrier protein reductase (InhA) -2NSD

While looking into the binding affinity it was found that Guineensine showed a significant binding affinity of -8.78Kcal/mol followed by β-asarone and Myristic acid with -5.73Kcal/mol and -5.62Kcal/mol. The standard drug Ethambutol showed a binding affinity of -4.41Kcal/mol. In depth analysis of the binding profile showed that Quercetin shared the highest similarity with the standard drug and formed 4 amino acid with Ethambutol with binding energy of -5.21Kcal/mol. The amino acid observed in common was ILE19, PHE 41, ILE 91 and PHE97. The compounds β-asarone, Gallic acid, Eugenol and Guineensine shared two active site amino acids in common. The amino acids PHE 41, ILE 91 and PHE 97 were found to be involved in most of the phytocompounds. Though the compound Guineensine had the highest binding energy while taking into account the interaction profile the amino acid found in common was only two but the compound Quercetin shared four amino acids in common though poses the binding energy of -5.21Kcal/mol. Similar docking studies was also been recently performed by Ghattas *et al* 2019, Ruswanto *et al* 2019, J.-F. Xu, *et al*. 2019 [25-27]. The significance of this study is the use of phytocompounds against potential enzyme InhA of Mycobacterium tuberculosis and a similar study performed by Sharma et al. 2019 [28] reported the *in silico* activity of Quercetin and depicted the binding affinity of -3.62Kcal/mol against InhA. In our study we have obtained a binding affinity of -5.21Kcal/mol which is much higher than Sharma et al. 2019 [28]. Thus among the screened and investigated ligands, Quercetin has been found to be an effective compound that could inhibit the potential enzyme InhA.

Conclusion

Tuberculosis is a world Based on the results of the computational analysis it was concluded that the compound's such as quercetin, gallic acid, eugenol, guineensine and β-asarone present in the formulation SC possess significant inhibition of Enoyl acyl carrier protein reductase enzyme present in mycobacterium tuberculosis thereby it was concluded that this formulation may have promising anti mycobacterial activity

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