



ANTITUBERCULAR EVALUATION OF *ANNONA SQUAMOSA* LEAVES ON H37RV STRAIN WITH COMPUTATIONAL APPROACH

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ABSTRACT:

Tuberculosis (TB) is an historic sickness that has affected mankind for greater than 4,000 years. TB generally affects the lungs but it could also have an effect on different elements of the body, which includes mind, intestines, kidneys, or the backbone. In pulmonary TB, it can reason signs, inclusive of continual cough, ache in the chest, hemoptysis, weak point or fatigue, weight loss, fever, and night time- sweats. Molecular docking has turned out to be more and more vital tool for drug discovery. In Molecular docking basic theories, which includes sampling algorithms and scoring features, are summarized. The variations in and overall performance of available docking software are also mentioned. Flexible receptor molecular docking processes, in particular the ones including backbone flexibility in receptors, are an undertaking for available docking methods. In present study Anti-Tubercular activity of *Annonasquamosa* leaves extract was evaluated by Luciferase enzyme and Alamar blue assay methods. The GC-MS Chromatogram done for ethyl acetate and aqueous fraction of *Annonasquamosa* leaf extract to identify the phytoconstituents. Molecular docking study was carried out to identify the plant constituents responsible for anti-tubercular activity by MVD and DNA gyrase enzyme was selected as target. The results revealed that the presence of 5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one, heptadecyl 2-methoxyacetate and 7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one have antitubercular activity against DNA Gyrase enzyme. They are safe and good absorb orally and they may be a promising lead molecule for the Antitubercular drug development.

KEYWORDS: Lipinski's rule of five, Drug likeness, Molecular docking, Luciferase reporter phage assay, Gas chromatography.

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I. INTRODUCTION

Tuberculosis is a lethal sickness caused by a bacterium called Mycobacterium tuberculosis. Tuberculosis (TB) is a typical irresistible ailment influencing lungs and other parts of body (Sakula A, 1982). Tuberculosis creates an alarming situation to the world, approximate 1.3 million deaths observed globally in 2020 due to tuberculosis. It is an air-borne infection, spread by individuals who have the malady, cough, sneeze, or spit; they move TB germs known as bacilli into the air (Chadha VK, 2009). India is one of the countries which actively working towards prevention of TB. The second biggest DOT (Directly Observed Treatment) program running in India to prevent infection of Tuberculosis. Treatment achievement rates have been significantly increased from 25% to 86% and TB passing rates have declined from 29% to 4% (Kaye K, 1996). Prolong use of anti-tubercular drugs for long time creating resistance so it's very essential to identify better targets site to achieve effective treatment at low dose. Over the last few decades, many attempts have been made to develop drugs to combat drug-resistant strains to control mycobacterial growth or enzymes associated with fatty acid synthesis and DNA replication have been targeted for new treatment options (Kochi A, 1991). Therefore, screening of natural products and their derivatives offers a new approach for the development of novel anti-TB drugs. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site (Gschwend DA, et al., 1996; Knechtel RM, et al., 1997). Molecular docking is useful for predicting both the strength and type of signal produced and used to identify potential drug molecules from a virtual library containing 461,397 compounds (Mezei M, 2003). *Annonasquamosa* belongs to *Annonaceae* family and contains 119 species. Most of the species belongs to *Annonaceae* family grow in tropical regions; e.g., the sour sop fruit tree (*Annona muricata*) is cultivated commercially and is widespread in the West Indies, North and South Americas, Africa, the

Pacific Islands, and Southeast Asia (NRCS, 2008). The leaves of the plants have been used as medicines by indigenous people for a wide range of disorders including parasitic infections, inflammation, diabetes, insecticide, anthelmintic (Pandya N, 2011). Bark is used as powerful astringent, antidiarrheal and vermifuge. Root, bark, leaves and stems gave isoquinoline, alkaloids. Two acetogenins, annonectin and isoannonectin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumors (Patel D, et al., 2008; Zahid M, et al., 2018). The leaves and stems also gave alkaloids dopamine, salsolinol and cocaine.

The present study aims to identify the lead molecules responsible for *In Vitro* Anti-Tubercular evaluation of *Annonasquamosa* leaves on H37Rv strain followed by Molecular docking.

II. MATERIALS AND METHODS

Plant authentication, collection and extraction

The leaves of *Annonasquamosa* were collected in the month of August, 2021 from nearby area of Guntur District, Andhra Pradesh, India. The plant part was authenticated by Dr. P. Satyanarayana Raju, M.Sc., M.Phil., Ph.D., and Plant taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. The leaves of the plant were tenderly evacuated, washed multiple times in faucet water lastly with refined water. After shade drying, the leaves were ground into fine powder by utilizing mixer (Prestige). The 500 g powder of leaves used for extraction in different solvents by Hot Continuous Extraction (Soxhlet) (Chin FS, et al., 2013). Solvent used in extraction arranged according to their polarity from non-polar to the polar like Petroleum ether, Ethyl acetate, Ethanol, Water by Soxhlet apparatus. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent (Wei Q, et al., 2013). The extract were carried out for Phytochemical evaluation.

Screening of anti-tubercular potential (*In-vitro*)

1. Luciferase enzyme assay method



A luciferase assay is used to determine if a protein can activate or repress the expression of a target gene (Parikh A, et al., 2013; Fu X, et al., 2015). With the help of luciferase assay, presence of the protein and the amount of gene product estimated but unable to determine whether the protein directly interacts with DNA or not. Antitubercular activity against *M. tuberculosis* H37Rv strain measured by embracing luciferase columnist phage test (**Figure 1**).

2. Alamar blue assay method

The anti- Mycobacterial activity of compounds were assessed against Mycobacterium tuberculosis using micro plate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, pink color was scored as growth (De Fries R, et al., 1995). The MIC was defined as lowest drug concentration which prevented the color change from blue to pink (**Figure 2**).

Identification of lead molecules by GC-MS

Agilent innovations 6890 N JEOL GC Mate II GC-MS model was used for Identification of components present in extracts and checked with database of National Institute Standard and Technology (NIST) (**Figure 3 and 4**) (Juszczak AM, et al., 2019).

Molecular docking studies

Molegro Virtual Docker selected for molecular docking which is a quick and adaptable

docking program that gives the most probable compliance of ligand authoritative to a macromolecule. The docking scoring capacity of Mol Dock is an expansion of the piece wise direct potential (PLP) including new hydrogen holding and electrostatic terms. To additionally improve docking precision, a re-positioning scoring capacity is presented, which distinguishes the most encouraging docking arrangement from the arrangements got by the docking calculation.

Preparation of ligand

The main focus for docking study was AChain (1F8i) of DHFrse, DNA Gyrase, and Isocitratelase compound. It is observed that PDB documents frequently have poor or missing assignments of express hydrogens, and the PDB record design can't oblige bond request data. Along these lines, legitimate bonds, bond requests, hybridization and charges were allotted utilizing the MVD.

The 3D structures of the phytoconstituents are retrieved from PubChem chemical databases and drawn using ChemDraw Ultra 8.0 software (Cambridge Soft Corporation, Cambridge.) and saved in .mol format after minimization of energy (**Figure 5-7**).

In silico assessment of Physicochemical and Pharmacokinetic properties of phytoconstituents

Identification of phytoconstituents were anticipated utilizing information warrior, adaptation 4.2.2 (Actelion Pharmaceuticals Ltd, Allschwil, Switzerland) and tabulate in Table 6.

In silico acute rat toxicity of phytoconstituents

LD50 value in rats was anticipated by GUSAR programming (Department for Bioinformatics, Institute of Biomedical Chemistry of the Russian Academy of Medical Sciences, Moscow, Russia) tabulate in Table 6.

III. RESULTS AND DISCUSSION

Phytochemical analysis showed the presence of different phytochemicals like alkaloids, glycosides, carbohydrates, cardiac glycosides, flavonoids, terpenoids, anthraquinones,



tannins, terpenoids, and phenolic compounds in the extracts.

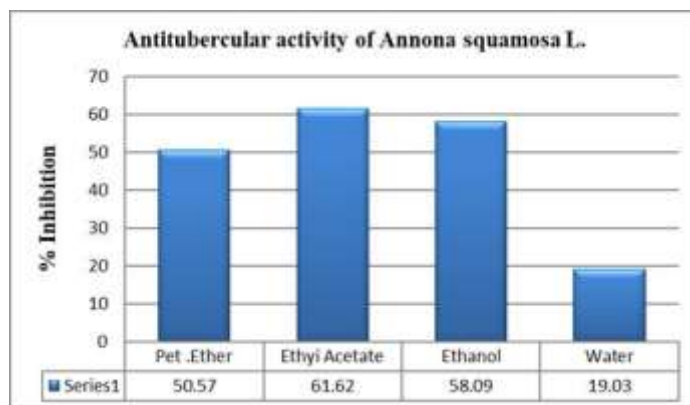


Fig-1: Effect of various extracts of *Annonasquamosa* leaves on *Mycobacteriumtuberculosis*(H37Rvstrain) byLuciferaseenzymeassay



Fig-2: Effect of various extracts of *Annonasquamosa* leaves on *Mycobacteriumtuberculosis*(H37Rvstrain)byAlamarblueassay(MICµg/ml).

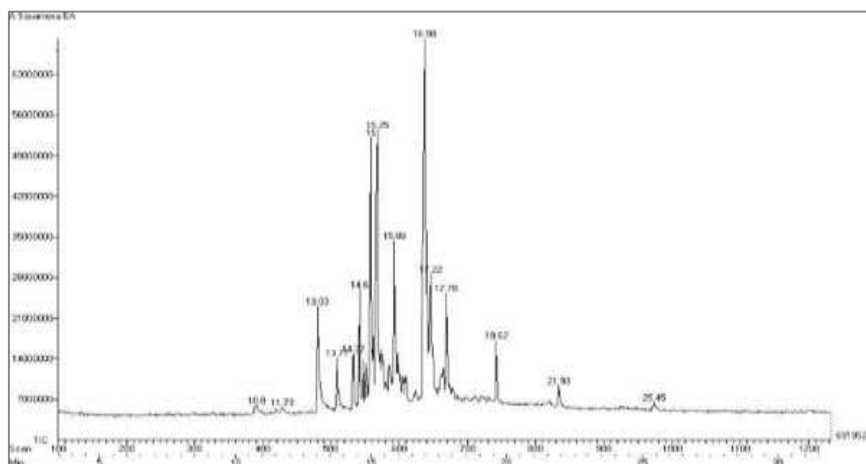


Fig-3: GC-MS Chromatogram obtained for ethyl acetate fraction of *Annonasquamosa* leaf extract

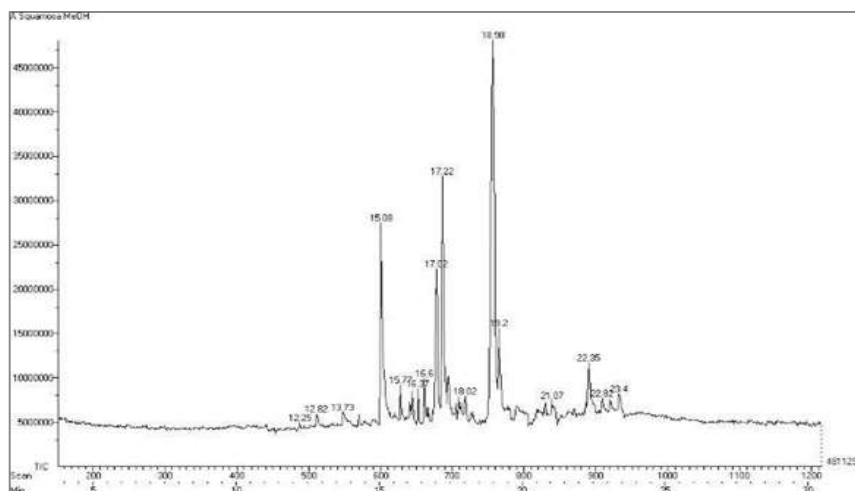


Fig-4:GC-MS Chromatogram obtained for aqueousfraction of *Annonasquamosa* leaf extract

Table 1. Phytochemical constituents identified by GC-MS analysis from EthylAcetateextractof*Annonasquamosa*leaves.

Mol.No	NameofMolecules	Retentiontime	Mass
EA1	3-isopropylbenzoicacid	11.73	164.0000
EA2	1a,4,8,8-tetramethyl-2,3,5,6,7,8,8a,8b-octahydro-1aH-cyclohepta[3,4]benzo[1,2-b]oxirene	13.77	220.0000
EA3	2-phenyl-4H-chromen-4-one	14.37	221.4019
EA4	2-phenylchroman-4,7-diol	14.6	242.0000
EA5	methylpalmitate	15.25	270.3043
EA6	Cycloicosane	15.88	280.5332
EA7	(E)-methyloctadec-8-enoate	16.98	296.5215
EA8	3-(2-((1-methyl-1H-imidazol-2-yl)thio)acetyl)-2H-chromen-2-one	17.78	300.0000
EA9	2,6-di-tert-butyl-4-(4-hydroxy-3,5-dimethylbenzyl)phenol	21.93	3340.0000
EA10	methyl 5,9,13,17-tetramethyloctadecanoate	25.45	354.0000
EA11	1-methyl-4-(propan-2-ylidene)cyclohexanol	10.8	154.0000
EA12	methyl16-methylheptadecanoate	17.22	298.4991
EA13	7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	19.62	336.6698
EA14	7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	15	268.4531
EA15	(Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	13.03	204.0000



Table 2. Phytochemical constituents identified by GC-MS analysis from Aqueousextract extract of *Annonasquamosaleaves*.

Mol.No	Nameofthe compounds	Mass	Retentiontime
A1	cinnamicacid	148.0000	12.25
A2	(Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	204.0000	12.82
A3	THUJOPSENE	204.0000	13.73
A4	tetradecanoicacid	228.0000	15.08
A5	3-hydroxy-2-phenyl-4H-chromen-4-one	1238.0000	15.77
A6	2-methyl-3-(o-tolyl)quinazolin-4(3H)-one	249.4531	16.37
A7	2-acetyl-3,5,8-trihydroxy-6-methoxynaphthalene-1,4-dione	278.5316	16.6
A8	9-Hexadecenoicacid,methylester	268.4643	17.02
A9	methylpalmitate	270.4853	17.22
A10	(E)-methyloctadec-16-enoate	294.0000	18.98
A11	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	302.9853	21.07
A12	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	328.0046	22.35
A13	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	335.8268	22.82
A14	heptadecyl2-methoxyacetate	343.0000	23.4
A15	11-methylenetricosane	298.5702	19.2
A16	1-(5-hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol	296.0000	18.02

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AssessmentofLigand–DNAgyraseenzymeinteractionbyMVD.

Molecular docking study was carried out to identify the plant constituents responsible for anti tubercular activity by MVD. DNA gyrase enzyme is selected as target protein and structural presentation of bacterial DNA gyrase (PDBID:4BAE). (a) surface structure of DNA gyrase, (b). grid generated around the binding pocket of ciprofloxacin. (c). 2d plot of ligand protein interaction profile by mvd. (d). visualization of methyl 5,9,13,17-tetramethyloctadecanoate-DNA gyrase, hydrogen bond interaction (Asp-79, Thr-169, Gly-83, Ile-84).



Table 3. Ligand – DNA gyrase enzyme interaction (MolDock Score, Rerank Score and H-Bond) of lead molecules of *Annona squamosa* leaves.

Mol.no	Name of the molecules	Target protein (PDBid)		
		bacterial DNA gyrase (4bae)		
		MolDock Score	Rerank Score	H-Bond
EA10	methyl 5,9,13,17-tetramethyloctadecanoate	-146.52	-115.2	0
EA8	3-(2-((1-methyl-1H-imidazol-2-yl)thio)acetyl)-2H-chromen-2-one	-134.66	-97.10	-6.83
A13	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	-130.87	-110.9	-12.7
A11	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-126.81	-96.09	-9.51
A16	1-(5-hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol	-124.80	-86.83	-1.53
EA13	7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-119.08	-87.66	-5.32
A14	heptadecyl 2-methoxyacetate	-114.30	-85.78	-2.5
EA7	(E)-methyl octadec-8-enoate	-111.25	-91.31	0
EA12	methyl 16-methylheptadecanoate	-110.87	-90.57	0
A10	(E)-methyl octadec-16-enoate	-110.53	-90.60	0
A9	methyl palmitate	-109.58	-90.68	0
EA5	methyl palmitate	-109.54	-90.68	0
EA9	2,6-di-tert-butyl-4-(4-hydroxy-3,5-dimethylbenzyl)phenol	-107.94	-54.75	-1.05
A12	methyl 16-methylheptadecanoate	-106.63	-74.42	0
EA3	2-phenyl-4H-chromen-4-one	-106.23	-89.59	-3.49
A6	2-methyl-3-(o-tolyl)quinazolin-4(3H)-one	-103.75	-84.99	-0.18
A15	11-methylenetricosane	-102.75	-28.49	0

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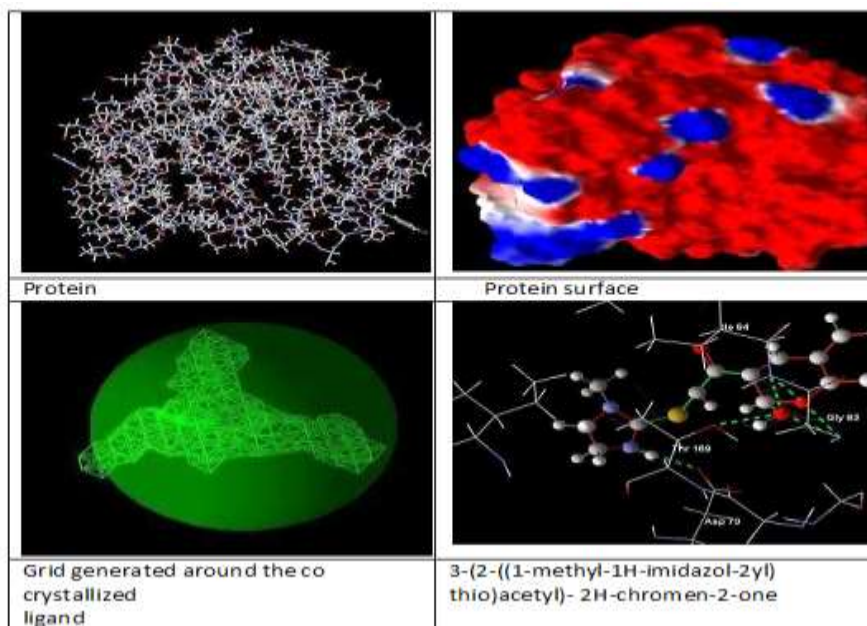


Fig-5: Ligand–DNAgyraseenzymeinteraction

Ligand–Dihydrofolatereductaseenzymeinteraction

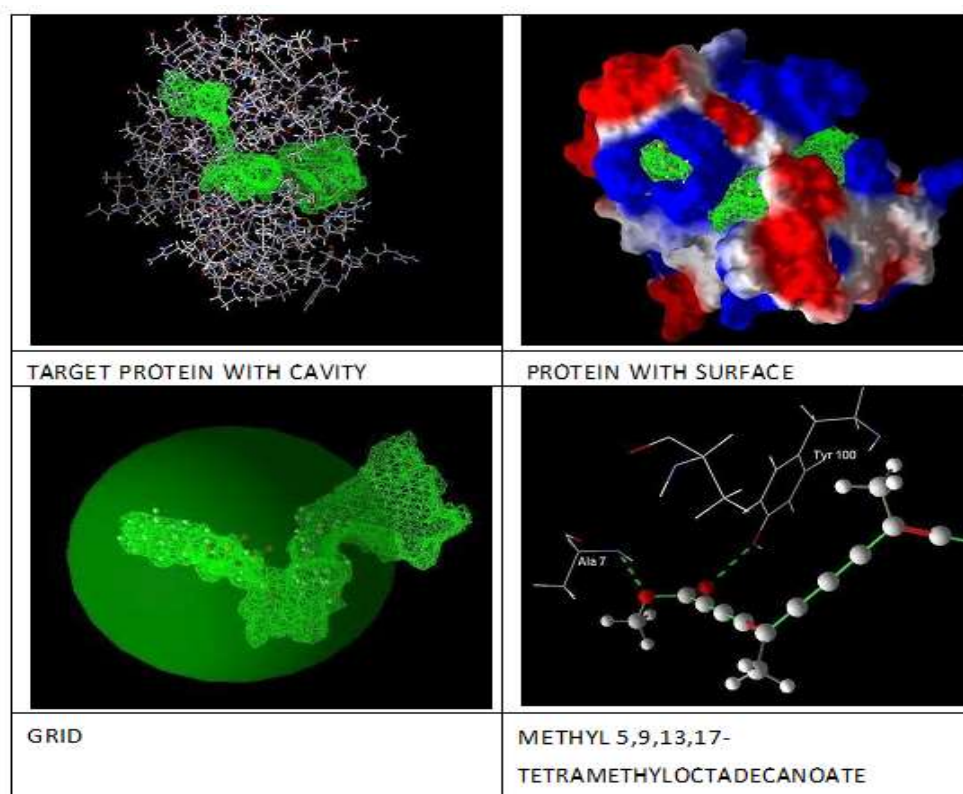
Structural presentation of bacterial DHFR (PDB ID:2CIG). (A) Surface structure of DHFR, (B). Grid generated around the binding pocket of INH. (c). 2D plot of lig and protein interaction profile by MVD. visualization of methyl 5,9,13,17-tetramethyloctadecanoate- DHFR, Hydrogen bond interaction (Asp-79,Thr-169,Gly-83,Lle-84).

Table 4. Ligand –Dihydrofolatereductase enzyme (Mol Dock Score, Rerank Score and H- Bond) of lead molecules of *Annonasquamosaleaves*.

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Mol.no	NameofMolecules	Targetprotein(PDBid)BacterialDHFR(2CIG)		
		MoldockScore	Rerankscore	H-bond
EA10.	methyl 5,9,13,17-tetramethyloctadecanoate	-180.43	-145.00	-4.74
A15.	11-methylenetricosane	-163.95	-129.73	0
A14	heptadecyl 2-methoxyacetate	-139.58	-111.92	-1.47
A11	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-138.19	-107.45	-8.52
A16	1-(5-hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol	-138.02	-94.00	-0.88
EA8	3-(2-((1-methyl-1H-imidazol-2-yl)thio)acetyl)-2H-chromen-2-one	-135.39	-109.57	-5.74
EA9	2,6-di-tert-butyl-4-(4-hydroxy-3,5-dimethylbenzyl)phenol	-135.36	-135.90	-2.5
EA13	7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-134.86	-104.48	-8.06
EA6	Cycloicosane	-132.41	-102.64	0
A13	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	-128.01	-86.23	-10.6

EA7	(E)-methyloctadec-8-enoate	-124.89	-98.88	-0.14
A10	(E)-methyloctadec-16-enoate	-123.46	-96.92	-0.13
EA14	2,3,14,17-tetrahydroxy-10,13-dimethyl2,3,4,5,9,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-6(10H)-one	-117.20	-87.72	-3.93
EA4	2-phenylchroman-4,7-diol	-116.52	-97.13	-6.76
A4	tetradecanoicacid	-112.24	-86.88	-0.24
EA3	2-phenyl-4H-chromen-4-one	-111.65	-89.70	-1.16
A2	(Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	-111.30	-88.56	0



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Fig-6: Ligand–Dihydrofolatereductaseenzyme

Ligand–isocitratelaseenzymeinteraction

Structural presentation of bacterial ICL (PDB ID:1F8I).(A) Surface structure of Isocitrate lyase (B). Grid generated around the binding pocket of succinic acid. (c). 2Dplot of ligand protein interaction profile by MVD. visualization of methyl 5,9,13,17-tetramethyloctadecanoate–Isocitratelase , Hydrogen bond interaction (Ala-7,Tyr-100).

Table5.Ligand–isocitratelaseenzymeInteraction(Mol DockScore,RerankScoreandH-Bond)ofleadmolecules of *Annonasquamosa*leaves.

Mol .No	NameofMolecules	TPROTEIN(PDBID)BACTERIALICL(1F8I)		
		MOLDOCKSCORE	RESCORE	H-BOND



EA10	methyl 5,9,13,17-tetramethyloctadecanoate	-112.90	-50.77	0
EA13	7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-103.79	-37.28	-18.05
A11	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	-101.65	-9.89	-22.27
A13	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	-90.7	6.49	-8.68
EA9	2,6-di-tert-butyl-4-(4-hydroxy-3,5-dimethylbenzyl)phenol	-88.84	13.10	-2.5
A16	1-(5-hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol	-88.47	-12.04	-7.47
EA1	3-isopropylbenzoic acid	-82.06	-70.56	-2.04
EA2	1a,4,8,8-tetramethyl-2,3,5,6,7,8,8a,8b-octahydro-1aH-cyclohepta[3,4]benzo[1,2-b]oxirene	-77.16	23.67	-3.18
EA15	(Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	-76.29	-14.44	0
A1	cinnamic acid	-75.47	-64.06	-7.69
A2	(Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	-74.91	-22.29	0
A7	2-acetyl-3,5,8-trihydroxy-6-methoxynaphthalene-1,4-dione	-67.08	-19.55	0.85
EA5	methylpalmitate	-64.81	65.71	-2.59
EA3	2-phenyl-4H-chromen-4-one	-58.90	-6.53	-3.27
EA14	2,3,14,17-tetrahydroxy-10,13-dimethyl-2,3,4,5,9,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-6(10H)-one	-54.67	160.73	-0.01
A4	tetradecanoic acid	-54.51	54.58	0
A6	2-methyl-3-(o-tolyl)quinazolin-4(3H)-one	-52.54	119.39	-1.99



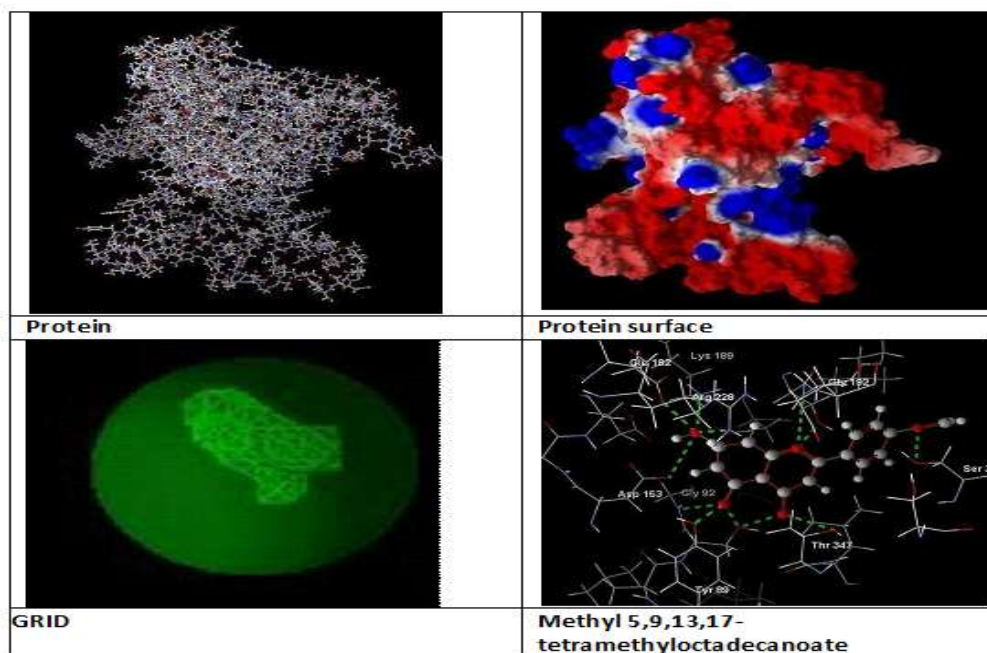


Fig-7: Ligand–isocitratelaseenzyme

***In silico* assessment of drug likeness of phytoconstituents:**

Physicochemical properties like molecular weight, logP, number of hydrogen bond acceptors, H-bond donors, rotatable bonds and total polar surface area were calculated using Data warrior and tabulated in Table 6 Drug likeness of phytoconstituents is based on their physicochemical properties and toxicity profile. These constituents obeyed Lipinski's rule of five and free from Mutagenicity, Tumorigenicity, Irritating effects and Reproductive effects as shown in Table 6. Drug likeness of these Phytoconstituents were calculated using Data

warrior version. Lipinski's rule describes molecular properties important for pharmacokinetics of a drug in the human body. logP in -0.4 to +5.6 ranges, Molecular weight from 180 to 500 Daltons, Not more than 5 H-bond donors and not more than 10 H-bond acceptors, 10 or fewer rotatable bonds and polar surface area equal to or less than 140 Å are predicted to have good oral bioavailability. They obey Lipinski's rule of five. It is applicable only to absorption by passive diffusion of compounds through cell membranes. Drugs absorbed through the active transport process are exceptions to this rule.



Table6.InsilicoassessmentofPhysicochemical,Pharmacokineticandtoxicitypropertiesofphytoconstituents

Mol.No	Name ofthe Molecules	Physicochemicalproperties					Pharmacokinetic properties				InsilicoToxicityassessment		
		Mol. formula	Mol. wt(gm/ml)	No .ofHBA	No.ofHBD	Log ^P	Absorbtion	BBB	BBB Permeation	Protein binding	Carcinogenicity	Mutagenicity	hERG inhibition
EA1	3-isopropylbenzoic acid	C7H6O2	122.12	2	1	1.44	High	1.61171	Yes	7.57	-ve	mutagen	Mediumrisk
EA2	1a,4,8,8- tetramethyl-2,3,5,6,7,8,8a,8b- octahydro-1aH-cyclohepta[3,4]benzo[1,2-b]oxirene	C15H24	204.3	0	0	4.25	Low	13.3193	Yes	100	+ve	mutagen	Medium risk
EA3	2-phenyl-4H-chromen-4-one	C15H10O2	222.24	2	0	3.18	High	2.2099	Yes	92.5	+ve	mutagen	Mediumrisk
EA4	2-phenylchroman-4,7-diol	C15H14O3	242.27	3	2	2.25	High	0.53251	Yes	90.0	-ve	mutagen	Mediumrisk
EA5	methylpalmitate	C17H34	270.45	2	0	5.54	High	8.21885	Yes	100	+ve	Non-mutagen	Low-risk
EA6	cycloicosane	C20H40	270.45	0	0	6.68	Low	20.4647	Yes	100	+ve	Non-mutagen	Mediumrisk
EA7	(E)-methyloctadec-8-enoate	C19H36O2	296.49	2	1	5.41	High	7.48966	Yes	100	+ve	mutagen	Low-risk
EA8	3-(2-((1-methyl-1H-imidazol-2-yl)thio)acetyl)-2H- chromen- 2-one	C14H16N2O3S	292.35	4	1	1.95	High	0.10995	No	16.1	+ve	mutagen	Mediumrisk

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EA9	2,6-di-tert-butyl-4-(4-hydroxy-3,5-dimethylbenzyl)phenol	C23H4 4O2	352.59	2	3	5.12	High	4.3452	Yes	100	+ve	Non- mutagen	Low- risk
E A10	methyl 5,9,13,17-tetramethyloctadecanoate	C23H4 6O2	352.59	2	3	5.12	Low	18.0974	No	100	+ve	Non- mutagen	Low risk
E A11	1-methyl-4-(propan-2-ylidene)cyclohexanol	C10H18O2	154.25	1	1	2.41	High	5.72576	Yes	32.4	-ve	mutagen	Low- risk
E A12	methyl 16- methylheptadecanoate	C19H3 8O2	298.50	2	0	6.20	High	15.3785	No	100	+ve	Non- mutagen	Low- risk
E A13	7-hydroxy- 2-(4-methoxyphenyl)-4H-chromen-4- one	C20H3 0O5	350.45	5	4	1.29	High	0.23080	No	48.2	-ve	Non- mutagen	Low risk
E A14	7-hydroxy- 2-(4-methoxyphenyl)-4H-chromen-4- one	C16H2 4O4	280.36	4	1	1.94	High	0.05044	Yes	70.0	-ve	mutagen	Low risk
E A15	(Z)-4,11,11-trimethyl-8-methylenebicyclo [7.2.0]undec-3-ene	C16H24	204.35	0	0	4.25	Low	13.3193	No	100	+ve	mutagen	Medium risk
A1	cinnamic acid	C9H14 O2	154.21	2	1	2.09	High	1.86487	Yes	60.8	-ve	mutagen	Medium risk
A2	(Z)-4,11,11-trimethyl-8- methylenebicyclo[7. 2 .0]undec-4-ene	C15H2 4	204.35	0	0	4.24	Low	13.3193	No	100	+ve	mutagen	Medium risk
A3	THUJOPSENE	C14H2 8O2	204.35	0	0	4.32	Low	12.9778	No	100	+ve	Non- mutagen	Medium risk
A4	tetradecanoicacid	C14H28O2	228.37	2	1	4.45	High	5.03596	Yes	100	+ve	mutagen	Low-risk
A5	3-hydroxy-2-phenyl-4H-chromen-4-one	C15H22O3	250.33	3	1	2.89	High	1.92664	Yes	100	+ve	mutagen	Low-risk
A6	2-methyl-3-(o-tolyl)quinazolin-4(3H)-one	C16H26N2 O	263.39	2	0	2.93	High	4.27333	Yes	88.9	+ve	mutagen	Low-risk

A7	2-acetyl-3,5,8-trihydroxy-6-methoxynaphthalene-1,4-dione	C13H16O7	284.26	7	3	-0.91	High	0.13549	No	32.9	+ve	mutagen	Lowrisk
A8	9- Hexadecenoicacid,methyl ester	C17H32O2	268.32	2	0	5.26	High	11.3756	Yes	100	-ve	Non-mutagen	Medium risk
A9	methylpalmitate	C17H32O2	270.45	2	0	5.54	High	13.9493	Yes	100	+ve	Non-mutagen	Low-risk
A10	(E)-methyloctadec-16-enoate	C16H24O5	296.36	5	2	1.18	High	0.04845	No	45.5	-ve	mutagen	Medium risk
A11	5,7-dihydroxy-2-(4-Methoxyphenyl)-4H-chromen-4-one	C15H22O7	314.33	7	5	-0.53	High	0.03247	No	29.0	-ve	Non-mutagen	Lowrisk
A12	5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	C20H40O3	328.53	3	0	6.10	High	11.211	No	100	-ve	Non-mutagen	Mediumrisk
A13	2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	C24H48	336.64	0	0	9.22	Low	27.4422	No	100	+ve	Non-mutagen	Mediumrisk
A14	heptadecyl2-methoxyacetate	C20H38NO4	355.51	5	2	1.92	High	0.32633	Yes	40.8	-ve	Non-mutagen	Low-risk
A15	11-methylenetricosane	C19H38O2	298.50	2	0	6.20	High	15.3785	No	100	+ve	Non-mutagen	Low-risk
A16	1-(5-hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol	C19H36O2	296.49	2	0	5.98	High	14.9483	No	100	+ve	Non-mutagen	Low-risk

IV. CONCLUSION

Ethyl acetate and Aqueous extract of Annonasquamosa leaves on In-vitro method demonstrates the anti tubercular activity. The presence of active principles 5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one, heptadecyl 2-methoxyacetate and 7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one may be responsible for this activity. The possible mechanism of action underlying the anti tubercular activity may be DNA Gyrase enzyme and DHFRase enzyme and ICLezyme inhibition. In silico drug likeness and toxicity prediction of 5,7-dihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one, heptadecyl 2-methoxyacetate and 7-hydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one reveals that they are safe and it can absorb orally and they may be a promising lead molecule for the Anti tubercular drug development.

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