SCREENING OF ANTIBACTERIAL FabH INHIBITORY POTENTIAL OF CONSTITUENTS PRESENTS IN *ANNONA MURICATA* THROUGH MOLECULAR DOCKING STUDIES

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ABSTRACT:

The flowering evergreen tree ***Annona muricata***, commonly known as soursop. Soursop is one plant that may have antibacterial properties. It has been observed that certain chemicals in soursop (***Annona muricata*** Linn) decrease the activity of the FabH enzyme (PDB code: 1MZS). The inhibitory potential of each of these test substances is still unknown and requires further investigation. This study investigated the potential of chemicals found in soursop as a FabH inhibitor for an antibacterial drug utilizing a molecular docking simulation and physicochemical and pharmacochemical descriptor analysis (using the Swiss ADME service). According to the findings, liriodenine has a binding energy of -8.1 kcal/mol and can reduce the activity of the FabH enzyme. Its physicochemical and pharmacochemical characteristics still fall within the bioavailability range of oral drugs. Additionally, Phe304, Ala216, Ile250, Ala246, Asn274, Leu189, Val212, and Met207 amino acids have been found to consistently occur in ligand-FabH interactions and are anticipated to be crucial in the mechanism of FabH inhibition, according to molecular interaction analysis.

KEYWORDS: ***Annona muricata***, ***E. coli***, FabH, Molecular docking.

INTRODUCTION:

The use of plants as medicine is a worldwide phenomenon; plants not only provide safe and cost-effective remedies, they are also available and accessible at affordable prices. The use of resources already available, forms the basic core of any public health practice and what is better than plants as medicine as they are associated with fewer side effects and no known resistance to microorganisms [1]. Ethno medicine may be broadly defined as the use of plants by humans as medicines but can be more accurately called ethnobotanic medicine [2].

Annona muricata is a flowering evergreen tree native to Mexico, Cuba, Central America and parts of India. The miracle tree as it is widely known as a natural cancer killer that is 10,000 times stronger than chemotherapy, based on these miraculous claims, the leaves of this plant were used as an extract at varying concentration as an antibacterial agent against oral pathogens [4]. The use of this plant in medicine has again come to fore as researchers are claiming it to have potential against common pathogen [5].

Soursop with its miraculous properties was used in this study with an intention to find newer use of these miracle plants. Ethno medicine has its roots in treatment of dental caries and periodontal disease. This has been well practiced in traditional medicine of various civilizations like Indian, Egyptian, Greek and Chinese [3]. Only about 6% have been screened for biologic activity and a reported 15% have been evaluated phytochemically [2]. This provides an avenue for newer search among plant kingdom for alternatives to traditional therapies and ***Annona muricata*** commonly called as Soursop is gaining worldwide acclaim for being a miracle tree in the field of cancer research and can pave way for research in many fields, including dentistry.

It has been briefly described by Ekins et al., 2007, that the history and development of a field that can be globally referred to as in silico pharmacology. This included the development of methods and databases, quantitative structure–activity relationships (QSARs), similarity searching, pharmacophores, homology models and other molecular modelling, machine learning, data mining, network analysis and data analysis tools that all use a computer. We have also previously introduced how some of these methods can be used for virtual ligand- and target-based screening and virtual affinity profiling. It will be greatly expanding on the applications of these methods to many different target proteins and complex properties, and discuss the pharmacological space covered by some of these in silico efforts. In the process, we will detail the success of in silico methods at identifying new pharmacologically active molecules for many targets and highlight the resulting enrichment factors when screening active drug like databases.

Drug Likeness Properties:

According to its older definition, "drug-likeness" evaluates qualitatively a molecule's potential to develop into an oral medication in terms of bioavailability. When development compounds were far enough along to be thought of as oral drug candidates, structural or physicochemical inspections were used to determine drug-likeness [16].

Drug-like and non-drug-like compounds can be distinguished using the Lipinski Rule of 5. It forecasts due to pharmacological similarity for molecules complying with 2 or more, there is a significant likelihood of success or failure.

1. Molecular mass less than 500 Dalton
2. High lipophilicity (expressed as LogP less than 5)
3. Less than 5 hydrogen bond donors
4. Less than 10 hydrogen bond acceptors
5. Topological Polar Surface Area <140

The violation of 2 or more of these conditions predicts a molecule as a non-orally available drug.

PLANT PROFILE:

Annona muricata L is a fruit tree, belonging to the annonaceae family. It is also known as soursop, graviola, and guanabana, is an evergreen plant with lot of traditional use. This plant is widely distributed in tropical and subtropical regions of the world.

TAXONOMICAL CLASSIFICATION:

- Kingdom : Plantae
- Division : Spermatophyta
- Subdivision : Angiospermae

- Class : Dicotyledanae
- Order : Polycarpiceae
- Family : Annonaceae
- Genus : ***Annona***
- Species : ***Annona muricata*** L

THERAPEUTIC USES:

The aerial parts of ***Annona muricata L*** (Soursop) have various functions such as the fruits have been widely used as food confectionaries; Like alkaloids, phenols and acetogenins there are more than 200 chemical compounds have been isolated and identified from ***Annona muricata L***.[7] In vivo, in vitro, and in silico studies have been taken from PubMed databases.[8] From 1981 to 2021 we have obtained 49 research article on ***Annona muricata L*** that shows, Anti-cancer activity-25%, Anti-ulcer activity-17%, Anti diabetic-14%, Anti protozoal-10%, Anti diarrohea-8%, Anti-bacterial activity-8%,Anti-viral activity-8%, Antihypertensive-6%, and Wound healing-4%.

MATERIALS AND METHOD:

Accerly's discovery studio viewer 4.0.1, Molinspiration, RCSB protein data bank, Online SMILES translator, MGL tools, Autodock 4.2, Python 2.7, PMV molecular viewer 1.5.6,Vision 1.5.6, Cygwin 64, Medchem designer 5.5, Pre ADMET.

The molecular descriptors were developed as rationally predictive and informative, for example, the Lipinski's Rule-of-Five. The better oral absorption of the ligands and drug likeness scores were constructed by getting information about the solubility, diffusion, Log P. molecular weight etc. This drug-likeness property, that is, the structural and physicochemical properties, supplements a pharmacophore without affecting the pharmacophore while giving the new compound sufficient pharmacokinetic behavior potential. Molinspiration software was used to evaluate the Lipinski's rule of five [34].

Lipinski's Rule of 5 determination

1) JAVA is required for using Molinspiration software.
2) Visit home page using http://www.molinspiration.com/.
3) Select "calculation of molecular properties of drug likeness".
4) Draw the structure of phytoconstituents in the active window.
5) Select the "calculates properties" and "predict bioactivity".
6) Save the properties displayed.

IN SILICO DOCKING STUDY ON FabH INHIBITORY ENZYME USING AUTODOCK 4.2

Enzyme identification for in silico docking

The crystal structure of FabH enzyme (PDB ID: 1MZS) [27]. was obtained from research laboratory for structural bioinformatics (RCBS) protein data bank. The refined protein was used further in silico studies.

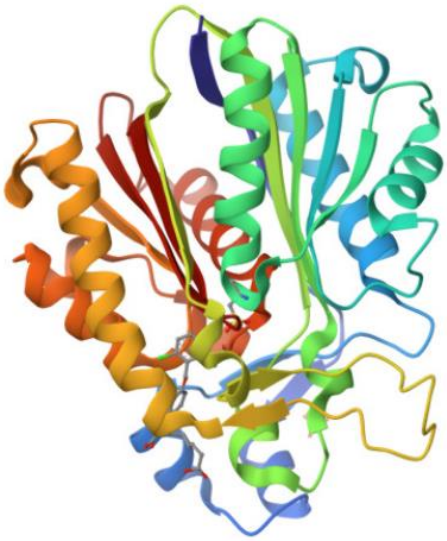

Fig. 1: **Refined structure of FabH enzyme from RCBS-PDB (1MZS)**

RESULT:

The docking analysis of various ligands against the FabH enzyme provides crucial insights into their binding affinities and potential antibacterial properties. Among the studied ligands, **Liriodenine** exhibited the highest binding affinity with a binding energy of **-8.1 kcal/mol** and an inhibition constant of **1.15 µM**, suggesting strong interaction with the FabH enzyme when comparing with the standard **Ciprofloxacin**. Similarly, **Xylopine (-7.86 kcal/mol, 1.73 µM), Annonaine (-7.73 kcal/mol, 2.17 µM), and Coreximine (-7.35 kcal/mol, 4.09 µM)** also displayed promising docking scores, indicating their potential role as antibacterial agents. On the other hand, compounds such as **Vitamin C (-4.36 kcal/mol, 633.9 µM) and Gallic acid (-5.9 kcal/mol, 5.25 mM)** showed weaker binding affinities, suggesting lower potential for FabH inhibition.

The differences in binding energies and inhibition constants can be attributed to variations in ligand-protein interactions, including hydrogen bonding, hydrophobic interactions, and electrostatic forces. In addition, **ligands like Diadezine (-7.33 kcal/mol, 4.26 µM) and Coronine (-7.19 kcal/mol, 5.38 µM)** demonstrated considerable binding potential, reinforcing the importance of flavonoid and alkaloid compounds in antibacterial activity. The variation in **RMSD values**, which **range from 26.57 to 56.13**, indicates different levels of conformational stability in docking poses, impacting the precision of ligand binding predictions. Overall, the docking results suggest that certain bioactive compounds from ***Annona muricata*** have strong interactions with FabH, which could be further explored in in vitro and in vivo studies to validate their antibacterial efficacy**.**

Table 1: **Docking results of various phytoconstituents with FabH inhibitory potential enzyme**

Name of the ligand	Binding Energy (kcal/mol)	Inhibition Constant	Intermolecular Energy	Internal energy	Torsional energy	RMSD Value
Coreximine	-7.35	4.1 μM	-8.54	-1.49	1.19	42.88
Annonaine	-7.73	2.2 μM	-7.73	0	0	38.35
Coronine	-7.19	5.4 μM	-8.69	-0.57	1.49	42.96
Xylopine	-7.86	1.7 μM	-8.16	-0.05	0.81	43.66
Liriodenine	-8.10	1.2 μM	-8.11	0	0	42.96
Gallic acid	-5.90	5.3mM	-4.61	-1.88	1.49	46.94
Isoboldine	-7.21	5.2 μM	-8.41	-1.53	1.19	40.25
Isolaureline	-7.34	4.2 μM	-7.63	-0.05	0.3	29.72
Vitamin C	-4.36	633.9 μM	-6.15	-2.43	1.76	56.13
Feruloylquinic acid	-7.30	34.5 μM	-6.81	-1.69	1.49	42.36
Diadezine	-7.33	4.3 μM	-8.22	-0.21	0.89	43.38
Ciprofloxacin	-6.26	25.7 μM	-7.46	-0.02	1.19	54.45

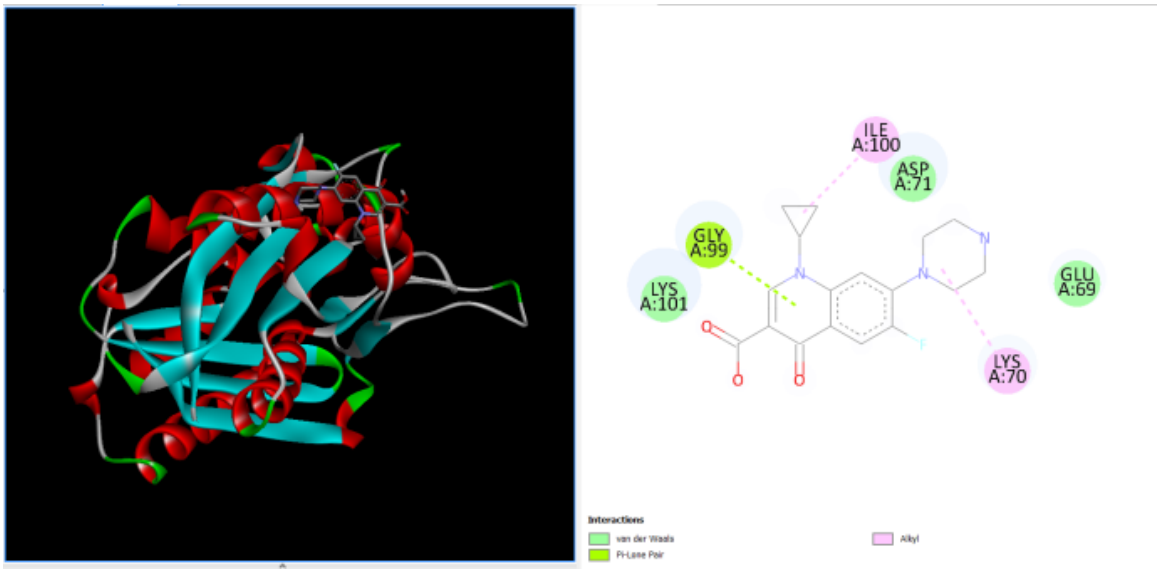

Fig. 2: STANDARD DRUG: CIPROFLOXACIN (-6.26)

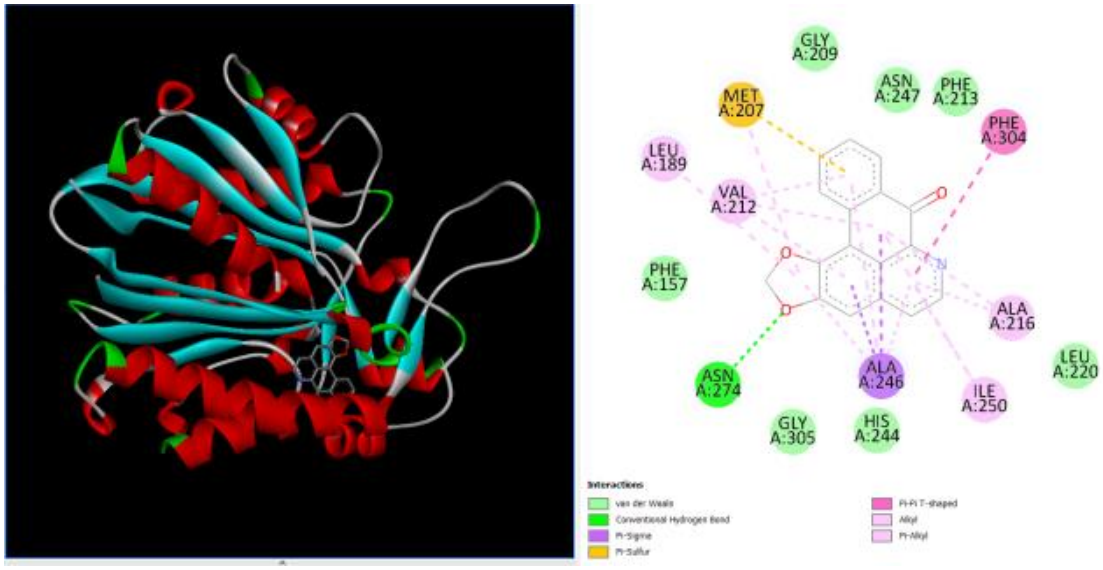

Fig. 3: HIGHEST BINDING ENERGY COMPOUND: LIRIODININE (-8.1)

Table 2: ADME PARAMETERS

S. No	Name of the Ligand	Diff Coef	M logP	S + logP	S + logD	M. Wt	M. No	TPSA	HBDH	Bioavailability Score	Synthetic Accessibility
1	Anomuricin	0.705	2.492	2.734	2.064	329.399	5	59.95	2	0.55	3.30
2	Glycitein	0.829	1.546	2.348	2.322	284.27	5	79.9	2	0.55	2.95
3	Xylopine	0.804	2.674	2.99	2.02	295.34	4	39.72	1	0.55	3.50
4	Caffeic Acid	1.061	0.994	1.262	-1.395	180.161	4	77.76	3	0.56	1.81
5	Catechin	0.829	0.757	0.775	0.746	290.274	6	110.38	5	0.55	3.50
6	Swainsonine	1.061	-0.643	-1.104	-1.732	173.213	4	63.93	3	0.55	3.15
7	N-Methylcoclaurine	0.741	2.779	2.552	2.219	299.372	4	52.93	2	0.55	2.92
8	Atherosperminine	0.705	3.503	4.626	2.864	309.411	3	21.7	0	0.55	2.09
9	Nornuciferine	0.782	2.808	3.381	2.255	281.357	3	30.49	1	0.55	3.35
10	Annonamine	0.741	-0.133	-0.95	-2.063	296.392	3	29.46	1	0.55	3.50
11	Argentinine	0.731	3.786	3.93	2.321	295.384	3	32.7	1	0.55	1.86
12	Isoloureline	0.771	2.907	3.372	2.799	309.367	4	30.93	0	0.55	3.61
13	Reticuline	0.705	2.492	2.742	2.366	329.399	5	62.16	2	0.55	3.52
14	Isoboldine	0.731	2.492	2.729	2.176	327.383	5	62.16	2	0.55	3.55
15	Robinetin	0.842	-0.235	2.056	1.724	302.242	7	131.36	5	0.55	3.21
16	Gentisic Acid	1.21	0.697	1.59	-1.867	154.123	4	77.76	3	0.56	1.10
17	DMDP	1.114	-2.117	-2.404	-2.769	163.175	5	92.95	5	0.55	2.95
18	Remerine	0.816	3.214	3.308	2.695	279.341	3	21.7	0	0.55	3.47
19	Anonaine	0.856	2.977	2.878	1.927	265.314	3	30.49	1	0.55	3.36
20	Feruloylquinic Acid	0.705	-0.72	0.008	-2.02	368.343	9	153.75	5	0.11	4.25
21	Daidzein	0.885	1.305	2.575	2.538	254.244	4	70.67	2	0.55	2.79
22	Epicatechin	0.829	0.757	0.775	0.746	290.274	6	110.38	5	0.55	3.50
23	Taxifolin	0.829	-0.081	0.952	0.618	304.258	7	127.45	5	0.55	3.51
24	Muricinine	0.76	2.26	2.492	1.752	313.356	5	70.95	3	0.55	3.52
25	Anomurine	0.682	2.208	3.174	2.198	343.426	5	48.95	1	0.55	3.30

26	DMJ	1.114	-2.117	-2.407	-3.021	163.175	5	92.95	5	0.55	2.95
27	Asimilobin	0.816	3.082	2.734	1.864	267.33	3	41.49	2	0.17	7.27
28	Coreximine	0.731	2.492	2.778	2.703	327.383	5	62.16	2	0.55	3.32
29	Gallic Acid	1.176	0.178	0.682	-2.652	170.123	5	97.99	4	0.56	1.22
30	Liriodenine	0.885	2.472	3.131	3.131	275.265	4	48.42	0	0.55	2.77
31	Isoferulic Acid	0.992	1.298	1.605	-1.063	194.188	4	66.76	2	0.85	1.90`x
32	Emodine	0.87	1.372	2.315	2.123	270.243	5	94.83	3	0.55	2.57
33	Coclaurine	0.771	2.542	2.135	1.453	285.345	4	61.72	3	0.55	2.78
34	Coronin	0.885	0.337	1.886	1.886	260.248	5	61.81	0	0.55	3.16
35	Fisetin	0.856	0.525	2.201	2.079	286.243	6	111.13	4	0.55	3.16
36	Cinnamic Acid	1.114	1.591	2.177	-0.519	148.162	2	37.3	1	0.85	1.67
37	Casuarine	1.014	-2.202	-2.285	-2.32	205.212	6	104.39	5	0.55	3.41
38	Morine	0.842	-0.235	1.915	1.413	302.242	7	131.36	5	0.55	3.25
39	Vitamin C	1.176	-2.224	-1.638	-1.633	176.127	6	107.22	4	0.56	3.47
40	DNJ	1.114	-2.117	-2.407	-3.021	163.175	5	92.95	5	0.55	2.95
41	Ciprofloxacin	0.755	0.588	-0.806	-0.826	331.349	6	74.57	2	0.55	2.51

DISCUSSION AND CONCLUSION:

Molinspiration software serves as a powerful tool in cheminformatics and computational drug discovery, providing essential features for molecular analysis, bioactivity prediction, and virtual screening. Its application in molecular docking studies has been instrumental in identifying potential drug candidates from natural sources, such as ***Annona muricata***.

The docking studies on FabH enzyme suggest that bioactive compounds from ***Annona muricata*** may possess antibacterial properties, warranting further in-vitro and in-vivo validation. The integration of in-silico ADMET predictions enhances the selection process by ensuring drug-likeness and safety profiles. Thus, computational approaches such as molecular docking and ADMET profiling play a crucial role in modern drug discovery, significantly reducing the time and resources required for experimental validation. Future research should focus on optimizing the identified lead compounds, conducting experimental assays to validate their efficacy, and exploring their potential as novel antibacterial agents.

The combination of computational and experimental methods will provide a more comprehensive understanding of the therapeutic potential of ***Annona muricata*** and other natural sources in drug discovery.

ADMET properties of a compound deals with its absorption, distribution, metabolism, excretion and toxicity in and through the human body. To determine biological activity and toxicity of compounds ADMET property of the phytochemicals were determined. ADMET constitutes the pharmacokinetic profile of a drug molecule which is very essential in evaluating its pharmacodynamic activities.

Drugs were withdrawn at the different stages of the clinical trials and from the market during the post-marketing surveillance (phase 4) owing to have poor ADMET properties and adverse events which are directly or indirectly associated with the molecular structure of the drugs. Therefore, in silico prediction of ADMET properties plays an important role during the lead identification and optimisations. Molinspiration software were used to predict ADMET properties like human intestinal absorption, blood brain barrier penetration, Caco-2 permeability, Madin-Dary canine kidney, Human Intestinal Absorption etc [9].

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