SCREENING OF BIOACTIVE COMPOUNDS IN *ABUTILON INDICUM* LEAVES WITH THE DIFFERENT DISEASES BY USING MOLECULAR DOCKING

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ABSTRACT

Abutilon indicum is (commonly known as Thuthi) a medicinal plant that belongs to the Malvaceae family, which is allotted for a duration of a wide variety of tropical and subtropical areas and has been used for more than a few issues in regular medicine. Its leaves, root, and bark has enormous medicinal value, and are used as an aphrodisiac, anti-diabetic, and diuretic. The leaf extract of Abutilon indicum is used to treat piles. Abutilon indicum(leaves and root) extract was tested to treat different diseases. The phytochemical screening was also carried out to find various phytoconstituents present in the Abutilon indicum. The ethanolic extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis for the identification of compounds present in the leaves sample. The identified compounds will be docked with different diseases (Tuberculosis, Leprosy, and Gonnorrhoeae).

Keywords: *Abutilon indicum*, phytochemical screening, Tuberculosis, Leprosy, Gonorrhoea.

1. INTRODUCTION

Abutilon indicum is one of the medicinal plants in the family Malvaceae.*Abutilon indicum* is popularly called Thuthi Keerai in Tamil Nadu. It also has health benefits and uses. *The Abutilon indicum* plant is very much used in Siddha medicines [1-4]. All the parts of that plant are used as medicine, and this plant is well known for its ability to treat all kinds of piles. Thuthi leaves are extremely beneficial for piles. It is used locally for high fevers, colds, tuberculosis, bronchitis, mumps, diabetes, hernias, haemorrhoids, diarrhoea, and various types of worm infections. *A. indicum* leaves are used in the treatment of toothache, lumbago, antifertility, and liver disorders. Bark and root are used as antidiabetics, aphrodisiacs, nervine tonics, and diuretics [5].

In 2020, 10 million people were off worldwide (TB), 5.6 million men, 3.3 million women, and 1.1 million children. TB is the 13th leadingcause of death and the second-leading infectious killer after COVID-19. (WHO) It is a contagious disease caused by *Mycobacterium tuberculosis*. It usually attacks the lungs, although it can also spread to other parts of the body, and is transmitted through coughing and sneezing. The simplicity with which TB infection spreads, for instance, by inhalation of droplets nuclei 2–5 mm in diameter containing as few as 1–3 bacilli has helped to sustain this scourge at current levels [6-7].

Globally 2020, 1,27,558 new leprosy cases were detected. It includes 8,629 children below 15 years. Leprosy affects humanity and it causes two to three million people to disable permanently. *Mycobacterium lepraeis* an etiologic agent, identified by the Norwegian doctor Gerhard Hansen, causes a chronic infectious disease called leprosy or Hansen's disease.. For ages, it is considered an incurable disease. Nerve damage, disfiguring skin sores, and progressive debilitation are some of the characteristics of this disease [8].

In,2018 United States showed that1.6 million new gonococcal infections occur and more than half occur among young people aged 15-24. Gonnorrhoeae is the second most commonly reported bacterial sexually transmitted infection in the United States. Gonorrhoeae is caused by the microorganism, *Neisseria gonorrhoeae*, and is one of the most common sexually transmitted infections in developing countries.Globally, there were an estimated 78 million cases of gonorrhoeae in 2012 [9-10].

2. METHODOLOGY

The fresh leaves were collected from Devipattinam, Ramanathapuram(district), Tamilnadu, India. Leaves were separated from the *Abutilon indicum* plant and then washed with water and then dried under shade at room temperature for 7 days. The dried leaves were grounded into a fine powder using the blender mixture. The extraction was carried out by using a solvent of ethanol.

Ten gram of dried finely powdered leaves sample was taken in 100ml of the ethanolic extract by dissolving the overnight. After, the extracts were magnetic stirred over 1-2 hours and then filtered using No.1 Whatman filter paper and stored in an airtight container for further analysis

Phytochemical screening: Qualitative analysis of extract was carried out to determine the presence of various bioactive compounds using the standard qualitative procedure.

Determination and screening of bioactive compounds by using GC-MS method: Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography Mass Spectroscopy (GCMS). GC-MS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio. The GC-MS analysis of extract of *Abutilon Indicum* leaves was carried out using Agilent GC 7890 with triple axis 5975 MS detector. The capillary column was Agilient HP-5MS (30 m x 250 μm x 0.25 μm) composed of 5% phenyl methyl silox. The initial oven temperature was 55 C for 0 min which was raised at rate of 10 C/min upto 200C for 0 min and then at rate of 5 C/min upto 260C for the hold time of 5 min. The injector volume was 10 μl. The Helium gas used as a carrier with constant flow rate of 1 ml/min with split ratio of 10:1. The MS operating conditions were; source temperature 250C (max-300C), quad temperature 150C (max-200C), solvent delay time of 3 min. Compounds were identified in terms of Rt values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed pharmacological activities.

Studies of *abutilon indicum* leaves and roots with three main protein: Bioactive compounds from leaves and root substances which belong to *Abutilon Indicum* were obtained. Those compounds were revealed by the GCMS analysis

Preparation of protein structure: The crystal of Three main protein structure was retrieved from Protein Data Bank RCSB PDB (http://www.rcsb.org/pdb/home/home.do)Then the PDB format of protein was subjected for docking studies.

Lipinski's rule: Lipinski's rule of 5 was developed by Christopher A. Lipinski in 1997, this rule was also called as Pfizer's rule of five or simply the rule of five (R05). This rule was developed to set "draggability" guidelines. In the drug discovery setting, the rule of 5 predicts that poor physiochemical and structural properties within certain ranges [12].

Preparation of target protein structure: LSR2 is the target protein against the phytocompounds identified from *Abutilon indicum*. The protein LSR2 is responsible for Tuberculosis. The three-dimensional crystal structure of LSR2 (PDB ID:4EIP). **3AFQ** is the target protein against the phytocompounds identified from *Abutilon indicum*. The protein 3AFQ is responsible for Leprosy. The three-dimensional crystal structure of 3AFQ (PDB ID:3AFQ). **6JTI** is the target protein against the phytocompounds identified from *Abutilonindicum*. The protein 6JTI is responsible for Gonnorrhoeae. The three-dimensional crystal structure of 6JTI (PDB ID:6JTI).

Molecular docking: Among the 55 ligands were used against the protein **4EIP,3AFQ,6JTI**. These compounds have been studied. Protein and ligands were converted into special file format PDBQT. Ligand preparation included the following steps (i) addition of hydrogen atoms, (ii) neutralization of the charge groups and (iii) removal of any miscellaneous structures from the ligand. Prepared and optimized structures of ligands were used for docking simulation. Grid values for protein the grid point set at 80 x 60 x 95. Before starting

the docking study, Vina wizard software was used to perform molecular docking in PyRx virtual screening tool. Discovery studio visualizer I was used to examine the docking poses of the complexes.

3. RESULT AND DISCUSSION

Phytochemical analysis of ethanolic extracts of *Abutilon Indicum* leaves indicated the presence of bioactive compounds. Reports were revealed the identification of major chemical constituents such as Quinones, Carbohydrates, Cardiac glycosides, Proteins, Amino acids, Steriods, Phytosterol, Saponins, Carboxylic acid, Fixed oils, Flavonoids are present. Comparing this paper, *Abutilon Indicum* is a huge phytochemical reservoir of various biologically active phytoconstituents such as carbohydrates, steroids, glycosides, flavonoids, tannins and Phenolic compounds.

The docking studies against **4E1P** revealed in GC-MS analysis of 2 bioactive Compounds showed the docking binding energy of (-6.8, -6.2) Kcal/mol respectively have good Tuberculosis activity against 4E1P [13].

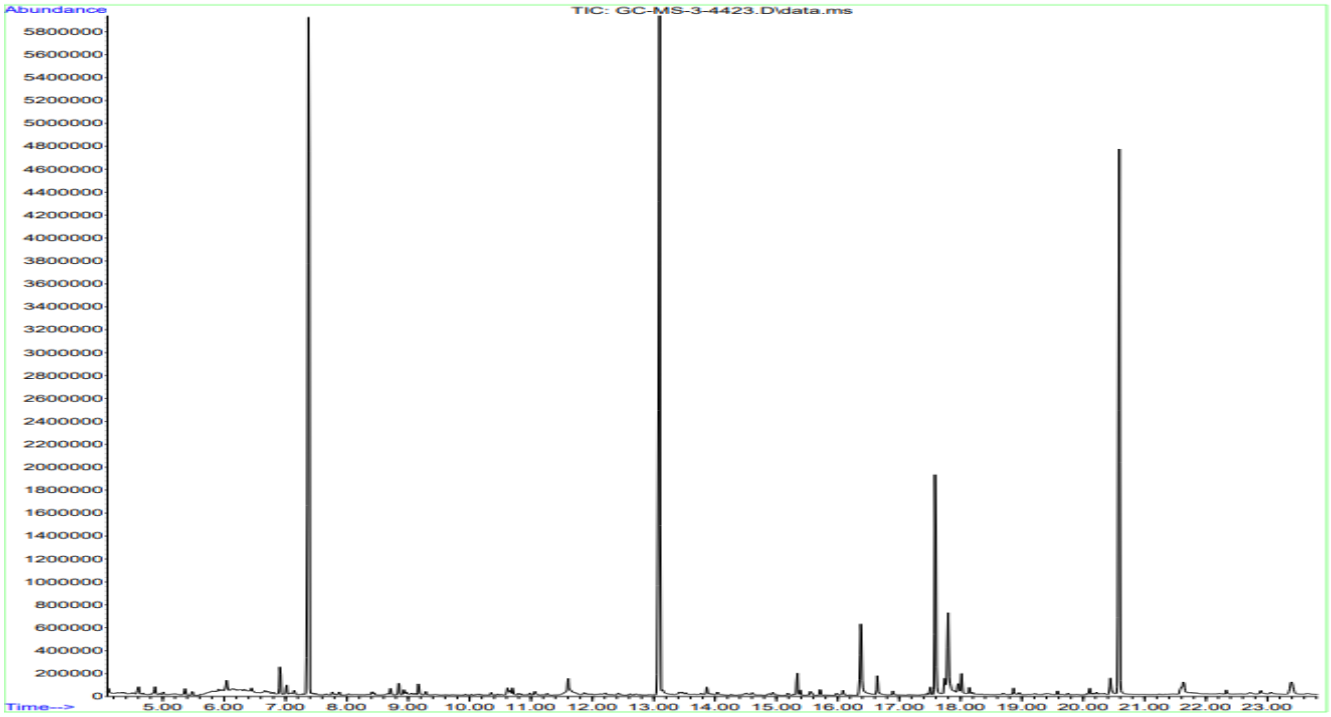
The docking studies against **3AFQ** revealed in GC-MS analysis of 4 bioactive Compounds showed the docking binding energy of (-8.8, -6.6, -8, -7.5) Kcal/mol respectively have good Leprosy activity against 3AFQ [14].

The docking studies against **6JTI** revealed in GC-MS analysis of 7 bioactive Compounds showed the docking binding energy of (-8.9,-7.4,-6.2,-6.3,-6.9,-6.6,-8.6) Kcal/mol respectively have good Gonnorrhoeae activity against 6JTI [15-16].

Table 1: Phytochemical screening(Leaves)

S.No	Phytochemical test	Results
1	Alkaloids	-
2	Phenols	-
3	Coumarins	-
4	Terpenoids	-
5	Quinones	+
6	Anthraquinones	-
7	Tannins	-
8	Phlobatannins	-
9	Carbohydrates	+
10	Glycosides	-
11	Cardiac glycosides	+
12	Proteins	-
13	Aminoacids	+
14	Steroids	+
15	Phytosteroids	+
16	Saponins	+
17	Acids	-
18	Carbocyclic acids	+

19	Fixed oils	+
20	Fats	-
21	Gums and Mucilages	-
22	Flavonoids	+



Graph 1: Gas Chromatography-Mass spectrometry chromatogram of ethanol extract of *Abutilon Indicum* (Leaves)

Molucular docking

4EIP(Tuberculosis): Bioactive compounds of *Abutilon Indicum* leaves against 4EIP Protein with the best Binding affinity.

Table 2: Pubchem ID, Binding Affinity of 4 EIP Protein with Ligands

PROTEIN	PUBCHEM ID	COMPOUNDS	BINDING AFFINITY
4EIP	73170	alpha-Amyrin	-6.8
	66831472	Thiophene,2,5-dihydro-	-6.2

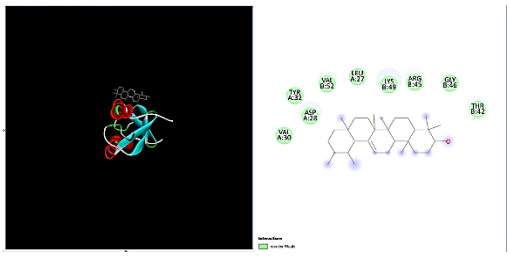


Figure 1:2D&3D interaction of protein 4E1P with alpha-amyrin

3AFQ (Leprosy): Bioactive compounds of *Abutilon Indicum* leaves against 3AFQ Protein with best Binding affinity.

Table 3: Pubchem Id, Binding Affinity Of 3afq Protein With Ligands

PROTEIN	PUBCHEM ID	COMPOUNDS	BINDING AFFINITY
3AFQ	73170	alpha-Amyrin	-8.8
	66831472	Thiophene, 2,5-dihydro-	-8
	22215476	Methyl3,4ethylidene-alpha-D- galactopyranoside	-7.5
	612809	9-Phenyl-5H-benzocycloheptene	-6.6

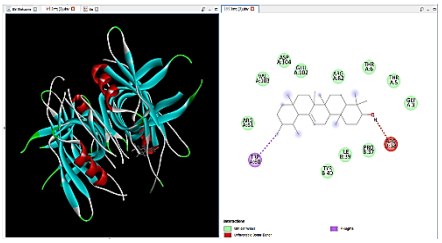


Figure 2:2D&3D interaction of protein 3AFQ with alpha-Amyrin

6JTI (GONNORRHOEAE): Bioactive compounds of *Abutilon Indicum* leaves against 6JTI Protein with best Binding affinity

Table 4: Pubchem ID, Binding Affinity of 6JTI Protein with Ligands

PROTEIN	PUBCHEM ID	COMPOUNDS	BINDING AFFINITY
6JTI	73170	alpha-Amyrin	-8.9
	66831472	Thiophene, 2,5-dihydro-	-8.6
	612809	9-Phenyl-5H-benzocycloheptene	-7.4
	6432313	Naphthalene, decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1.alpha.,4a.alpha.,7.alpha.,8a.beta.)]-	-6.9
	152273	Phenol, 3-(2-hydroxyethoxy)-	-6.6
	612605	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	-6.3
	5367325	7,10,13-Hexadecatrienoic acid, methyl ester	-6.2

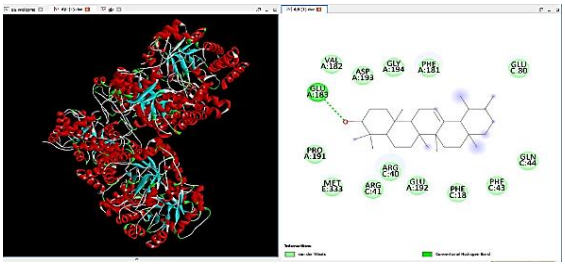


Figure 3:2D&3D interaction of protein 6JTI with alpha-Amyrin

Lipinski Rule Of *Abutilon indicum* (Leaves) Bioactive Compounds Analysed Using admetSAR

Table 5: Lipinski rule of *Abutilon Indicum*(leaveas) bioactive compounds analysed using admet SAR

S.No	Compound	Molecular weight	Log p<5	H Bond donor<5	H-Bond acceptor<10	No. of violation
1	Methyl 3,4-ethylidene-alpha-D- galactopyranoside	220.22	-1.2	2	6	0
2	Thiophene, 2,5-dihydro-	470.6	1.1	0	1	0
3	Phenol, 3-(2-hydroxyethoxy)-	154.16	0.9	0	3	0
4	Naphthalene, decahydro-1,4a-dimethyl-7-(1-methyl ethyl)-, [1S-(1.alpha., 4a.alpha.,7.alpha.,8a.beta.)]-	208.38	6.3	0	0	1
5	Phthalic acid, cycloheptylisohexyl ester	346.5	6.6	0	4	1
6	7,10,13-Hexadecatrienoic acid, methyl ester	264.4	5.1	0	2	1
7	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	218.33	3.2	0	1	0
8	alpha-Amyrin	426.7	9	1	1	1
9	9-Phenyl-5H-benzocycloheptene	218.29	4.7	0	0	0

4. SUMMARY AND CONCLUSION

Abutilon indicum leaves were collected from Devipattinam, and an ethanolic extract was prepared. Phytochemical screening was done on ethanolic extract. Docking studies were done by using compounds obtained from ethanolic extract (GC-MS analysis) with proteins **4EIP, 3AFQ, and 6JTI.** Phytochemical analysis of Ethanol extracts of *Abutilon indicum* leaves indicated the presence of bioactive compounds. The results obtained from the phytochemical analysis had shown similarities with the literature.

Molecular docking has become an increasingly important tool for drug discovery. The completion of the human genome project has resulted in an increasing number of new therapeutic targets for drug discovery. From the molecular docking studies, we conclude that identified novel bioactive ligands of *Abutilon indicum* leaves extract were checked for their three different diseases(Tuberculosis, Leprosy, Gonorrhoeae) using the PyRx-Python prescription (version 0.8) virtual screening tool.

The properties of the root of *Abutilon indicum* were studied *in silico* level. In the future, in vitro, and in vivo studies can be done to develop or transform *Abutilon indicum* into nanogel and nano tablet as a painkiller and drug to cure targeted diseases (tuberculosis, gonorrhoea, leprosy).

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