ANTI-TUBERCULOSIS ACTIVITY OF *FICUS RACEMOSA LINN.* PLANT EXTRACT, ITS HR-LCMS AND MOLECULAR DOCKING-BASED SCREENING FOR THE ACTIVE PHYTOCHEMICAL LIGAND METABOLITE: AN *IN-VITRO* AND *IN-SILICO* APPROACH

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Abstract-

Background: Many new mutated strains of TB are reported resistant to the long duration first and second-line treatment regimen and the toxicity of existing drugs is also more. Therefore, there is a need for alternative antitubercular molecules to overcome the above problems. Many plant-derived phytochemicals and their derivatives have been reported for antitubercular activity by binding with various mycobacterium receptor sites.

Objective: To prepare an aqueous extract of the plant *Ficus racemosa linn.*, testing its antitubercular activity and characterization using the chromatographic method. The detailed profiling of phytoconstituents present in the aqueous extract using the suitable analytical technique, and this data to be processed for in-silico molecular docking to identify the most likely metabolite showing anti-tubercular activity.

Method: The solid aqueous extract was prepared using the maceration process and dried at 45^0 C in an oven and characterized using an RP-HPLC (Shimadzu prominence Japan, Pump - LC-20AD, detector - SPD-M20A PDA, column - Phenomenex Luna 5u C18 at 35^0 C, 1 ml/min flow rate) for two different polarity mobile phase solvent systems (Acetonitrile and Methanol). The HR-LCMS (Agilent technologies, LC Q-TOF-MS, Version B5125.3) technique was employed to identify aqueous extract metabolites. For the docking study, two anti-tubercular receptors 3IFZ, and 5IBG were selected and structures were obtained from online rcsb.org/pdb/ website., its format was changed to PDBQt (Discovery studio software) and used. Out of seventy, sixty-five metabolites 3D structures were prepared from the Pubchem database. The PyRx docking software tool was used for the docking study and results were visualized using BIO-VIA Discovery Studio 2021. Drug likeness was tested using Lipinski's rule of five on the molsoft website.

Results: Prepared aqueous extract shows 100% inhibitory action on *Mycobacterium tuberculosis* using Lowenstein-Jenson inoculation medium. The developed RP-HPLC chromatograms give many characteristic peaks for prepared crude extract. The results of the docking study report Ohioensin-A, Triflusulfuron-methyl, Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside, 7 Hydroxymethyl -12 -methylbenz[a]anthracene sulfate, and 4,4-Difluoropregn-5-ene-3,20-dione were highest values for Receptor-Ligand binding affinity. The metabolite Ohioensin-A shows more Receptor-Ligand binding affinity for both receptors but poor drug-likeness as compared to Triflusulfuron-methyl and shows better results among all other metabolites. Docking results were visualized in 3D structures and seen as significant binding areas. The ADMET profile predictions for selected five ligands suggest promising reports for its drug development ability.

Conclusion: Aqueous Ficus racemosa Linn extract was tested for anti-tubercular activity and characterized using an HPLC chromatogram. Metabolites reported in the HR-LCMS study were screened for most active metabolites using a docking study and five probable drug leads were reported along with their drug likeness and ADMET profile.

Keywords: HR-LCMS analysis, *Ficus racemosa linn.*, Anti-tuberculosis activity, molecular docking, mycobacterium receptors.

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Introduction –

Tuberculosis (TB) is an airborne, curable, fatal disease and patients require a long time of treatment with multiple antibiotics. The need for newer active lead compounds and drug molecules in the management of tuberculosis has increased as resistance is reported by *Mycobacterium tuberculosis* strains with the existing first-line and second-line anti-TB drugs(Bansal et al., 2016). Plants are the major source of new drugs as it gives many advantages over synthetic chemicals and bout 25% of market available drugs are pure isolated compounds from plant or their chemical derivatives. Plants are used for antimicrobial activity in various ancient systems of medicine(Deep et al., 2013; Mohiuddin and Lia, 2020). Many plant-derived biologically active phytochemical compounds are promisingly effective against *Mycobacterium tuberculosis* by targeting various receptor binding sites(Mi et al., 2022). *Ficus racemose* (Family: Moraceae, Genus: Ficus, Species: Racemosa) is commonly available in various regions in India as well as many parts of the world and is reported for its uses in various human diseases. Since ancient times, various parts of this plant as leaf, bark, fruit, and latex have been used in Ayurveda to cure or prevent different diseases(Rengarajan and Yaacob, 2016). A variety of pharmacological activities like hypoglycemic, hypolipidemic, antitussive, wound healing, hepatoprotective, antibacterial, etc. have been reported due to the presence of various flavonoids and polyphenols from its leaf, bark, unripe fruit, galls, and latex (Bagyalakshmi et al., 2019; Elhawary et al., 2018; Yadav et al., 2015). *Ficus racemose* leaf extracts using various solvent reports antimicrobial activity against both Gram-positive and Gram-negative organisms and some wound pathogens (Bagyalakshmi et al., 2019; Mandal et al., 2000).

Considering the various promising antimicrobial reports for *Ficus racemose* leaf extracts, leaf aqueous extract was investigated for antitubercular activity and then for detailed phytochemical investigation using HR-LCMS and prediction of most likely antitubercular phytoconstituents screening by using molecular docking study(Alsulami and Gull, 2018; Lokesh ST et al., 2019). The two mycobacterium receptors, DNA gyrase and MTB CYP121 are docked with HR-LCMS metabolites to understand their binding properties. The results of *in-silico* binding properties of reported plant metabolites with selected antitubercular receptors will explore the likeliness for reported antitubercular activity among the obtained metabolites. Using HR-LCMS and molecular docking data, this study will report the novel lead antitubercular molecules among the various detected phytoconstituents of aqueous plant extract of *Ficus racemose* (Shivakumar et al., 2018).

Materials And Method

Plant material collection (Alsulami and Gull, 2018; Ogunlowo et al., 2013)

The fresh leaves of *Ficus Racemosa Linn.* plants were collected from the local area of Pandharpur, Dist- Solapur (Maharashtra) India in the month of August. Plant authentication was done from ICMR-NITM Nehru Nagar Belagavi by submitting required plant part samples (Herbarium No. RMRC - 1638).

Sample Preparation- Collected fresh plant leaves were cleaned with distilled water and dried in the room, without exposure to sunlight. The sample was taken for air drying for 8-10 days until constant weight, crushed using an electric grinder to get fine powder for maximum dissolution in the selected solvent, and kept in an airtight plastic bag until further use in the extraction process(Kanase et al., 2018).

Aqueous extraction of dried leaf powder- Water was selected as a solvent for extraction because phytoconstituents present in the aqueous extract usually do not possess solubility and bioavailability problems in its use as a lead molecule in the drug development process. The 250 gm of dried fine powder was weighed accurately and added into 1000 ml distilled water in a large beaker, mixed well, covered, and kept for 24 hrs. in a thermo-shaker. The liquid portion was filtered through muslin cloth and then Whatman filter paper (No. 1) in a large beaker and subjected to air drying in a hot air oven at 45^0 C until dried completely and stored in a tightly closed glass bottle at 4^0 C until further use(Azmir et al., 2013; Im et al., 2015).

Antitubercular activity using Lowenstein-Jenson (LJ) medium- –

The prepared aqueous extract was tested for anti-tuberculosis activity at STDC Nagpur for its inhibitory action on *Mycobacterium tuberculosis* using Lowenstein-Jenson (LJ) medium(Mi et al., 2022; Obulesu Gundala et al., 2022). The Lowenstein-Jenson medium is an egg-based glycerol-containing selective medium for the growth of different mycobacteria. The medium was completely dissolved, sterilized, and used for the test.

HPLC Chromatogram development –
Aqueous extract was subjected to develop a chromatogram using Shimadzu prominence RP-HPLC (LC-20AD, Japan), containing pump - LC-20AD, autosampler - SIL-20AC HT, detector - SPD-M20A PDA, column Phenomenex Luna 5u C18(2), column temperature 35^0 C and LC 1.25 solution software. Two suitable mobile phase Acetonitrile with Formic Acid (0.1%) in ratio10:90 v/v and Methanol with Formic Acid (0.1%) in ratio 50:50 were selected along with 1 ml/min flow rate, 15 min. run time and 10 µL injection volume(Bouzid et al., 2014; Kedar et al., 2022).

Profiling of Phytoconstituents using HR-LCMS technique (Noumi et al., 2020; Satpute S B and Vanmare D J, 2018; Shivakumar et al., 2018) –
The *Ficus Racemsoa Linn.* aqueous leaf extract was tested for metabolite profiling using HR-LCMS (Agilent technologies, LC Q-TOF-MS, Version B5125.3) analysis at SAIF laboratory, IIT Bombay, Powai, Mumbai, India. The plant metabolites were identified using its generated chromatogram data and comparing it with retention time and unique molecular mass fragmentation data available in the Metlin library available with IIT, Bombay. The method 30min_+ESI_ 11012021_MSMS.m, binary pump G4220B, elution solvent with a gradient system of 0.1% formic acid in water and acetonitrile 0.3 mL/min flow rate, gas flow 13 L/min, pressure 0.00-1200.00 bar, injection volume 5 µL, acquisition mode mass range 115-1100 m/z, gas temperature 250° C, and stop time 30 min. were applied for HR-LCMS analysis(Dhas et al., 2021).

Molecular Docking / In Silico Study (Elhawary et al., 2018; Sharma et al., 2022) –
Selection and preparation of receptor structures (Proteins) –
The two anti-tubercular target proteins (receptors) DNA gyrase receptor (PDB ID : 3IFZ), and MTB CYP121 receptor (PDB ID: 5IBG) are selected because they are involved in the general multiplication of *Mycobacterium tuberculosis* and not of a specific antitubercular drug(Adeniji et al., 2020, 2018). The 3IFZ is the crystal structure of the first part of the *Mycobacterium tuberculosis* DNA gyrase reaction core (breakage and reunion domain at 2.7 A resolution) and 5IBG is the Crystal structure *Mycobacterium tuberculosis* CYP121 in complex with inhibitor fragment 25b are available online.
Selected anti-tubercular target receptor proteins 3IFZ, and 5IBG structures were obtained from the online rcsb.org/pdb/ website, heteroatoms were removed, and the addition of polar hydrogens were performed and saved in PDB format (Figure 3). Using Discovery Studio software, the above format was changed into PDBQt and used.

Preparation of metabolites obtained in HR-LCMS analysis (ligands) for docking study –
A total of seventy metabolites are reported in HR-LCMS results and out of which sixty-five metabolites were taken as ligands for docking study to study its interaction with selected Mycobacterium tuberculosis receptor molecules (Table 1). Five metabolites were not included in the docking study as their data is not available in the online Pubchem compound database. The 3D structures were obtained from an online Pubchem compound database source (http://pubchem.ncbi.nlm.nih.gov). Discovery Studio was used for the conversion of these SDF files of all ligand molecules into useful PDB forms.
Mycobacterium receptor proteins and ligand docking – The online available docking software tool, PyRx (http://PyRx.sourceforge.net/) was downloaded and used for docking of selected receptors and ligands. For visualization of docking results, BIO-VIA Discovery Studio 2021 software was used.
Drug likeness calculations – To check drug-likeness conditions, scanning of all obtained ligands were carried out. Lipinski's rule of five was used for testing drug-likeness conditions on the website https://www.molsoft.com public domain data of molsoft was referred using the smiles format of phytoconstituents (Ligand). Lipinski's attribute's like number of hydrogen acceptors and donors, molecular weight, and partition coefficient log P were obtained from molsoft data(Bakht et al., 2010).

ADMET predictions – The online tool http://lmmd.ecust.edu.cn/admetsar2/ was used for predicting major pharmacokinetic ADMET (absorption, distribution, metabolism, elimination, and toxicity) parameters(Srivastava et al., 2020).

Results and discussion –
Plant material collection, preparation, and extraction - The proper collection of plant material and its processing is a very important step for separation and identification during the used method of plant secondary metabolite fingerprinting. Used maceration process for the preparation of crude extract using water gives black colored extract which becomes solid after drying in an oven at 45^0 C.
Weight of leaves sample before drying = 500 gm

Weight of leaves sample after drying = 16.43 gm
% Yield of crushed leaves powder = (16.43/500) X 100 = 3.29

Antitubercular activity testing –
The aqueous extract was tested for anti-tuberculosis activity at STDC Nagpur and reported 100% inhibitory action for *Mycobacterium tuberculosis* using a Lowenstein-Jenson inoculation medium.

HPLC Chromatogram development –
HPLC analysis was performed for *Ficus Racemosa Linn.* crude extract using Shimadzu prominence RP-HPLC (LC-20AD, Japan) to get chromatographic data using different solvent systems. This developed chromatogram gives information about number of present metabolites and characteristic chromatogram for this plant crude extract at specified chromatographic conditions. When mobile phase was acetonitrile-formic acid (0.1%) 10:90 v/v, the developed chromatogram shows characteristic peaks at RT 2.911, 4.456, 8.834, and 11.589 and for methanol-formic acid (0.1 %) 50:50 v/v at 2.730, 3.666, 7.575 (Figure 1).

Profiling of Phytoconstituents using HR-LCMS technique –
Metabolite profiling of crude aqueous leaf extract of *Ficus Racemsoa Linn.* was performed using the Q-TOF HR-LCMS method at the SAIF facility, IIT Bombay. This is high resolution liquid chromatography coupled with mass spectroscopy for identification of plant secondary metabolites depending on their retention time, MS/MS fragments m/z, type of metabolite, and reference plant metabolite database library. Both positive and negative ionization mode data were provided using MS and total seventy metabolites were reported and their molecular weight ranges from 125.17 to 498.4. The obtained chromatograms are reported in Figure 2. and all metabolites with found details are reported in Table No. 1.

Molecular Docking / In Silico Study - Selected mycobacterium receptor proteins and ligand docking –
For the molecular docking study, PyRx docking software was used for selected 3IFZ and 5IBG receptors and 65 ligands. Molecular docking was performed in which the active site of target receptors were docked with ligands. The molecular receptor-ligand interaction docking site is in center X: 4.6500, Y: 10.8564 and Z: 22.9358 and with dimensions X: 77.7144 Å, Y: 91.7745 Å and Z: 89.7579 Å. The outcomes of this docking study were reported as values of binding affinity and interaction of ligand with receptors. The Receptor-Ligand binding affinity is measured in kcal/mol and reported in Table No. 2.

The results for selected ligands shows that, DNA gyrase receptor (3IFZ) reports Triflusulfuron-methyl, Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside, and Ohioensin-A and for MTB CYP121 receptor (5IBG) Ohioensin-A, 7 Hydroxymethyl -12 -methylbenz[a]anthracene sulfate, and 4,4-Difluoropregn-5-ene-3,20-dione were the first three ligands which shows highest value for Receptor-Ligand binding affinity. The metabolite Ohioensin-A shows more Receptor-Ligand binding affinity both with DNA gyrase (3IFZ) and MTB CYP121 (5IBG) selected receptors.
Further the Limpinski rule of five were applied to all 65 metabolites to find its drug likeness. Limpinski parameters were compared for the metabolites which showed maximum binding with selected receptors. The drug-likeness score for the metabolites showing maximum binding energy was -0.40 and 0.73 for Ohioensin-A and Triflusulfuron-methyl respectively. This indicates that even though Ohioensin-A has more binding energy but has poor drug likeness as compared to Triflusulfuron-methyl. All other metabolites reported less binding energy and not complying the Limpinski rule of five in one or more parameters. Therefore with consideration of the docking study and Limpinski rule of five , Triflusulfuron-methyl is showing better results among all other metabolites for antitubercular activity. Table No 3. Finally, for visualization of docking results BIO-VIA Discovery Studio 2021 software was used and resultant 3D docking of both selected receptors with Ohioensin-A and Triflusulfuron-methyl is shown in Figure 3.

ADMET predictions –
Data of major pharmacokinetic parameters of the first three ligands for both the receptors which show the highest value for Receptor-Ligand binding affinity were taken from the online tool and used for interpretation for screening of better suitable active metabolite for antitubercular activity.
The obtained ADMET profile of the selected ligands based on the docking results is listed in the Table No. 4. All ligands predicted better human intestinal absorption except Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside. The ligand 4,4-Difluoropregn-5-ene-3,20-dione shows the highest

blood brain barrier and acute oral toxicity effect. The least carcinogenicity was predicted with the ligand 7 Hydroxymethyl -12 - methylbenz[a]anthracene sulfate.

Conclusion -

Using Lowenstein-Jenson inoculation medium, antitubercular activity for aqueous extract of *Ficus racemosa Linn*. was performed and reported 100% inhibitory action for *Mycobacterium tuberculosis*, and characteristic chromatogram were developed using mobile phase acetonitrile with Formic Acid (0.1%) in ratio10:90 v/v and Methanol with Formic Acid (0.1%) in ratio 50:50 at specified conditions. All the seventy phytoconstituents profiling were done using HR-LCMS present in aqueous extract. In order to find the probable antitubercular metabolite among all HR-LCMS reported metabolites, a molecular docking study was performed using the two most common antitubercular receptors DNA gyrase (3IFZ) and for MTB CYP121 (5IBG). The five most prominent metabolites Triflusulfuron-methyl, Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside, Ohioensin-A, 7 Hydroxymethyl -12 - methylbenz[a]anthracene sulfate, and 4,4-Difluoropregn-5-ene-3,20-dione were identified based on highest ligand-receptor binding affinity. Ohioensin A found more binding affinity for both the antitubercular study but had less drug-likeness score whereas triflusulfuron-methyl shows the highest extent of drug likeness score. From this study, we have identified the most probable *Ficus racemosa Linn.* metabolites that were responsible for antitubercular activity, themselves or their derivatives may be the promising leads in the antitubercular drug development.

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Figure

Figure 1. Ficus racemose aqueous extract chromatogram –
A) ACN FA (0.1) 10:90 v/v
B) Methanol FA (0.1) 50:50 v/v

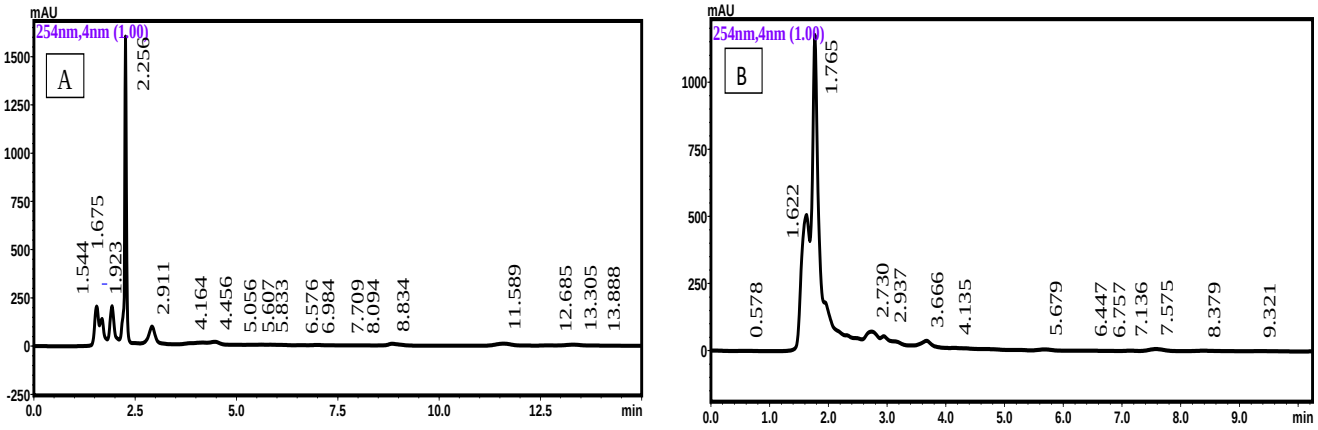


Figure 2. HR-LCMS Chromatogram of Ficus racemose aqueous extract

Sample Name	FR Met	**Position**	P1-C1	**Instrument Name**	QTOF	**User Name**	
Inj Vol	5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	FR-Met-ve.d	**ACQ Method**	30min_-ESI_11012021_	**Comment**		**Acquired Time**	8/29/2021 2:19:12 AM

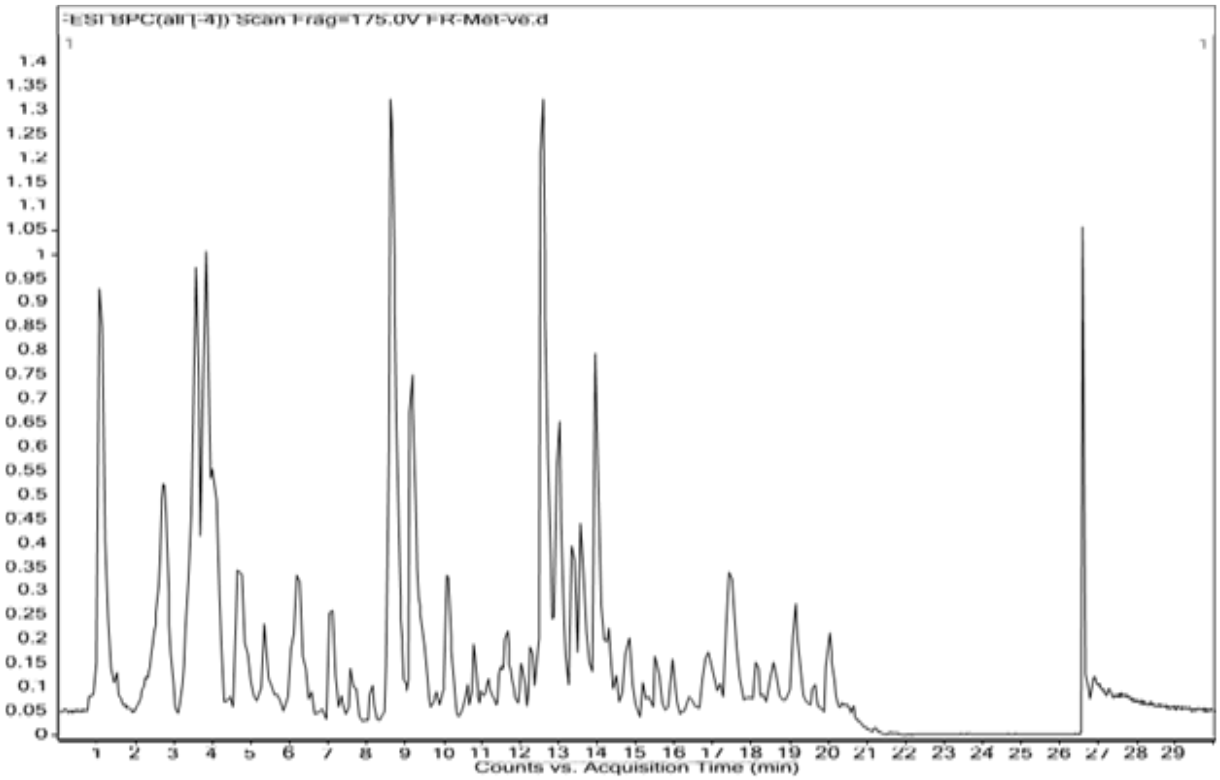
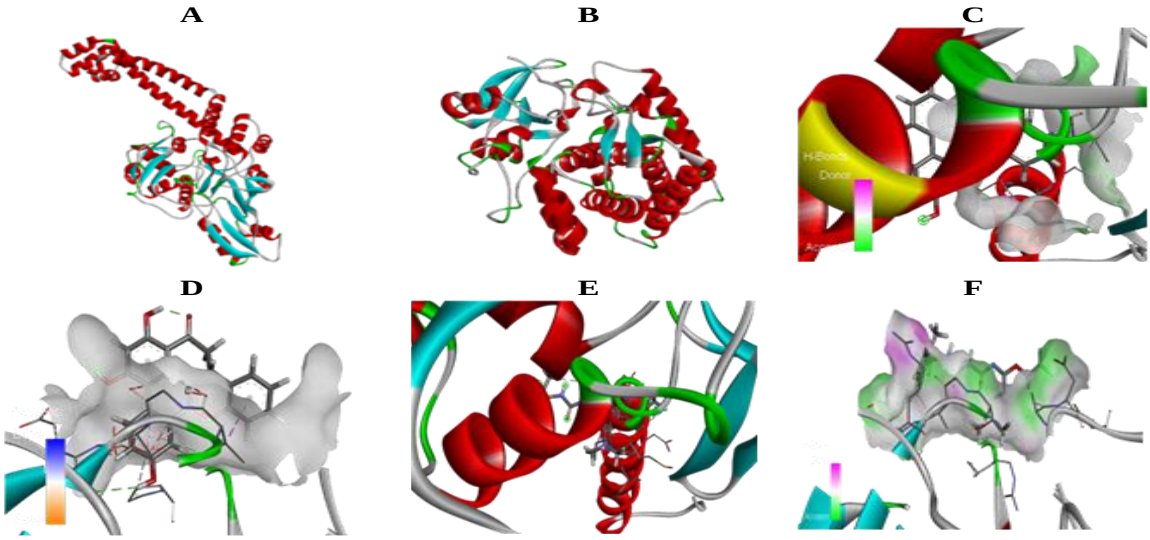


Figure 3. Images of Selected Receptors and Receptor Ligand interactions –
A) DNA gyrase receptor (3IFZ) -
B) MTB CYP121 receptor (5IBG) –
C) Interaction of 3IFZ and Ohioensin A –
D) Interaction of 5IBG and Ohioensin A –
E) Interaction of 3IFZ and Triflusulfuron-methyl
F) Interaction of 5IBG and Triflusulfuron-methyl



Tables

Table No 1 Identified secondary metabolite (Phytochemical) composition of Ficus Racemosa aqueous extract using the HR-LCMS technique

Sr No	Identified Compound Name	Formula	RT in Min	Mass	[M+H]+ (m/z)	[M+H]- (m/z)
1.	5-Hydroxydopamine	C8 H11 N O3	1.377	169.0727	170.0801	-
2.	1-[(5-Methyl-2- furanyl)methyl]pyrrolidine	C10 H15 N O	1.577	165.1145	166.1217	-
3.	Tebuconazole	C16 H22 Cl N3 O	1.898	307.1523	308.1594	-
4.	N-Methacrylylglycine methyl ester	C7 H11 N O3	2.212	157.0733	158.0805	-
5.	5,6,7,8-Tetrahydro-4- methylquinoline	C10 H13 N	2.509	147.1041	148.1114	-
6.	Ethyl N-ethylanthranilate	C11 H15 N O2	3.088	193.1094	194.1167	-
7.	Koenigicine	C20 H21 N O3	3.196	323.1473	324.1547	-
8.	Valyl-Tyrosine	C14 H20 N2 O4	3.609	280.142	281.1484	-
9.	DHAP(10:0)	C13 H25 O7 P	3.715	324.1311	325.1382	-
10.	1-Nitronaphthalene-5,6-oxide	C10 H7 N O3	3.867	189.0418	190.049	-
11.	Ethyl 2-furanpropionate	C9 H12 O3	3.936	168.0779	169.0852	-
12.	Carvyl propionate	C13 H20 O2	4.542	208.1454	209.1527	-
13.	3-Methylbutyl 2- furanbutanoate	C13 H20 O3	4.775	224.14	225.1473	-
14.	Rosuvastatin	C22 H28 F N3 O6 S	4.842	481.1569	482.1639	-
15.	2-Ethyl-5-imino-1-cyclopenten- 1-ol	C7 H11 N O	5.006	125.0833	126.0905	-
16.	Cyclopentolate	C17 H25 N O3	5.188	291.1824	292.1897	-
17.	2,4,6-Trimethyl-4-phenyl-1,3- dioxane	C13 H18 O2	5.336	206.1297	207.1369	-
18.	Capsaicin	C18 H27 N O3	5.553	305.1978	306.2052	-
19.	(+)-cis-5,6-Dihydro-5-hydroxy- 4-methoxy -6-(2-phenylethyl)- 2H-pyran-2-one	C14 H16 O4	5.783	248.1039	249.1111	-
20.	Dehydrovomifoliol	C13 H18 O3	5.859	222.1244	223.1317	-
21.	Isobutyl 2-furanpropionate	C11 H16 O3	5.936	196.1089	197.1161	-
22.	2,2,6,7-Tetramethylbicyclo[4.3.0]nona-1(9),4-dien-8-one	C13 H18 O	6.179	190.1343	191.1416	-
23.	3,5,8-Megastigmatrien-7-one	C13 H18 O	6.506	190.1344	191.1417	-
24.	Vestitone 7-glucoside	C22 H26 O9	6.65	434.1561	435.1634	-
25.	(9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid	C18 H26 O3	6.886	290.1871	291.1945	-
26.	Benzyl trans-2-methyl-2- butenoate	C12 H14 O2	7.202	190.0984	191.1057	-
27.	5-Methyl-2-phenyl-2-hexenal	C13 H16 O	7.744	188.1192	189.1264	-
28.	Austroinulin	C20 H34 O3	8.146	322.2497	323.2567	-
29.	3-Oxopregn-4-ene-20beta- carboxaldehyde dioxime	C22 H34 N2 O2	8.179	358.2706	359.2778	-
30.	Phendimetrazine	C12 H17 N O	8.24	191.1302	192.1376	-
31.	(10Z,14E,16E)-10,14,16-Octadecatrien-12-ynoic acid	C18 H26 O2	8.618	274.1919	275.1992	-
32.	[7]-Paradol	C18 H28 O3	8.686	292.2024	293.2097	-
33.	19-Noretiocholanolone	C18 H28 O2	8.761	276.2075	277.2147	-
34.	10-Oxo-11-octadecen-13-olide	C18 H30 O3	8.761	294.2181	295.2254	-
35.	D-Lysine	C6 H14 N2 O2	9.133	146.1089	147.1161	-
36.	Diethylpropion	C13 H19 N O	9.252	205.1467	206.1528	-

37.	(+)-Myrtenyl formate	C11 H16 O2	9.361	180.115	181.1212	-
38.	4,4-Difluoropregn-5-ene-3,20-dione	C21 H28 F2 O2	9.655	350.2057	351.2125	-
39.	[2,2-bis(2-methylpropoxy)ethyl]benzene	C16 H26 O2	9.897	250.1933	251.1991	-
40.	Noralfentanil	C16 H24 N2 O2	10.755	276.1838	277.1966	-
41.	(9Z,11R,12S,13S,15Z)-12,13-Epoxy-11-hydroxy-9,15-octadecadienoic acid	C18 H30 O4	11.31	310.2144	311.2205	-
42.	Lycocernuine	C16 H26 N2 O2	12.007	278.1994	279.2123	-
43.	Plumieride	C21 H26 O12	2.667	470.1378	-	515.136
44.	3'-Methoxyfukiic acid	C12 H14 O8	3.332	286.0655	-	285.0583
45.	Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside	C21 H22 O14	3.375	498.0963	-	497.0891
46.	Triflusulfuron-methyl	C17 H19 F3 N6 O6 S	3.4	492.0981	-	551.1124
47.	L-Homocystine	C8 H16 N2 O4 S2	3.663	268.055	-	327.0685
48.	3-propylmalic acid	C7 H12 O5	3.766	176.0656	-	175.0584
49.	Ohioensin-A	C23 H16 O5	3.937	372.1013	-	417.0995
50.	m-Coumaric acid	C9 H8 O3	4.595	164.0447	-	209.0429
51.	Furfural diethyl acetal	C9 H14 O3	4.784	170.0915	-	215.0896
52.	7-Hydroxymethyl-12-methylbenz[a]anthracene sulfate	C20 H16 O4 S	4.985	352.0754	-	411.0891
53.	Streptidine	C8 H18 N6 O4	5.621	262.1386	-	261.1312
54.	Nifedipine	C17 H18 N2 O6	5.914	346.113	-	345.1056
55.	3-Hydroxy-10'-apo-b,y- carotenal	C27 H36 O2	6.466	392.2735	-	391.2663
56.	N-[(5-Hydroxy-2-pyridinyl)methyl]adenosine	C16 H18 N6 O5	6.709	374.1324	-	433.1462
57.	9S,12S,13S-trihydroxy-10E- octadecenoic acid	C18 H34 O5	6.783	330.2366	-	389.2504
58.	10'-Apo-beta-caroten-10'-al	C27 H36 O	7.696	376.2787	-	375.2715
59.	6k-PGF1α-d4	C20 H30 D4 O6	7.885	374.2631	-	373.2559
60.	9S,11R,15S-trihydroxy-2,3- dinor-13E-prostaenoic acid- cyclo[8S,12R]	C18 H32 O5	8.483	328.2221	-	327.2144
61.	9,10-Dihydroxy-12,13-epoxyoctadecanoate	C18 H34 O5	8.784	330.2373	-	329.23
62.	Fortimicin KK1	C14 H30 N4 O7	8.826	366.2104	-	365.204
63.	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	C12 H20 O4	9.087	228.1335	-	227.1261
64.	Phloionolic acid	C18 H36 O5	9.403	332.2527	-	331.2455
65.	2R-hydroxy-stearic acid	C18 H36 O3	9.748	300.2628	-	359.2768
66.	Ricinoleic acid	C18 H34 O3	9.845	298.2471	-	357.2611
67.	5-Acetoxydihydrotheaespirane	C15 H26 O3	11.029	254.185	-	313.1988
68.	9Z-Octadecenedioic acid	C18 H32 O4	11.493	312.2267	-	311.2192
69.	LPA(0:0/18:0)	C21 H43 O7 P	13.888	438.2697	-	483.268
70.	Laserpitin	C25 H38 O7	16.171	450.2698	-	509.2836

Table No 2. Docking Results and Lipinski Rule of Five Data –

Sr No.	**Ligand Name**	**Binding Affinity (kcal/mol)**		**Lipinski Rule Parameters**				
		3IFZ	**5IBG**	**Mol. Weight**	**Lipophilicity (m LogP)**	**H-Bond Donar**	**H-Bond Accepter**	**Drug likeness score**
1.	5-Hydroxydopamine	-5.7	-5.8	169.18	-0.66	5	4	-1.01
2.	1-[(5-Methyl-2-furanyl)methyl]pyrrolidine	-4.7	-5.5	165.23	2.03	0	2	-0.73
3.	Tebuconazole	-6	-7.1	307.82	3.67	1	3	-0.07
4.	N-Methacrylylglycine methyl ester	-4.5	-5.3	143.14	-0.29	2	3	-1.41
5.	5,6,7,8-Tetrahydro-4-methylquinoline	-5.8	-6.3	147.22	2.82	0	1	-0.2
6.	Ethyl N-ethylanthranilate	-5.6	-5.7	193.24	2.84	1	2	-0.58
7.	Koenigicine	-7.1	-8.6	323.4	4.27	1	3	-1.06
8.	Valyl-Tyrosine	-6.4	-7.1	280.32	-2.42	5	5	-0.37
9.	DHAP(10:0)	-5.1	-6.4	324.31	2.78	2	7	-0.69
10.	1-Nitronaphthalene-5,6-oxide	-5.9	-6.5	189.17	1.49	0	3	-1.93
11.	Ethyl 2-furanpropionate	-4.7	-5.6	168.19	2.11	0	3	-1.41
12.	Carvyl propionate	-5.7	-6.2	208.3	4.26	0	2	-1.03
13.	3-Methylbutyl 2-furanbutanoate	-4.8	-6	224.3	3.63	0	3	-0.85
14.	Rosuvastatin	-7	-8.5	481.5	2.95	3	8	1.00

15.	2-Ethyl-5-imino-1-cyclopenten-1-ol	-4.3	-5.3	125.17	0.81	2	2	-0.77
16.	Cyclopentolate	-5.8	-6.4	291.4	2.3	1	4	2.07
17.	2,4,6-Trimethyl-4-phenyl-1,3-dioxane	-6.1	-6.5	206.28	2.95	0	2	-0.88
18.	Capsaicin	-6.8	-7.6	305.4	3.19	2	3	0.14
19.	(+)-cis-5,6-Dihydro-5-hydroxy-4-methoxy-6-(2-phenylethyl)-2H-pyran-2-one	-6.9	-7.4	248.27	1.79	1	4	-0.48
20.	Dehydrovomifoliol	-5.4	-6.4	222.28	1.38	1	3	-1.14
21.	Isobutyl 2-furanpropionate	-4.7	-6	196.24	3.01	0	3	-0.78
22.	2,2,6,7-Tetramethylbicyclo[4.3.0]nona-1(9),4-dien-8-one	-5.7	-6.8	190.28	2.74	0	1	-0.74
23.	3,5,8-Megastigmatrien-7-one	-6.4	-7.3	176.3	4.64	0	0	-0.41
24.	(9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid	-5.4	-7	290.4	4.35	1	3	0.65
25.	Benzyl trans-2-methyl-2-butenoate	-5.5	-6.4	190.24	2.91	0	2	-1.46
26.	5-Methyl-2-phenyl-2-hexenal	-5.8	-6	188.26	3.79	0	1	-0.97
27.	Austroinulin	-6.3	-7.6	322.5	4.15	3	3	-0.28
28.	3-Oxopregn-4-ene-20beta-carboxaldehyde dioxime	-8	-9.2	358.5	3.47	2	4	0.23
29.	Phendimetrazine	-5.8	-6.5	191.27	2.32	0	2	0.74
30.	(10Z,14E,16E)-10,14,16-Octadecatrien-12-ynoic acid	-5.2	-7	274.4	5.22	1	2	0.03
31.	[7]-Paradol	-5.5	-6.9	292.4	4.75	1	3	-0.53
32.	19-Noretiocholanolone	-6.8	-8	276.4	3.1	1	2	0.21
33.	10-Oxo-11-octadecen-13-olide	-6.6	-7.8	294.4	5.36	0	3	-1.26
34.	D-Lysine	-4.5	-4.8	146.19	-3.42	5	4	-0.71
35.	Diethylpropion	-5	-5.9	205.3	2.16	0	2	0.56
36.	(+)-Myrtenyl formate	-5.2	-5.7	180.24	3.3	0	2	-1.25
37.	4,4-Difluoropregn-5-ene-3,20-dione	-7.9	-9.6	350.4	4.27	0	2	0.18
38.	[2,2-bis(2-methylpropoxy)ethyl]benzene	-5.5	-6.3	250.38	4.74	0	2	-0.67
39.	Noralfentanil	-5.5	-5.8	276.37	1.05	1	3	0.17
40.	(9Z,11R,12S,13S,15Z)-12,13-Epoxy-11-hydroxy-9,15-octadecadienoic acid	-6	-6.7	310.4	4.24	2	4	-0.08
41.	Lycocernuine	-7.3	-7.5	278.39	1.75	1	3	-0.60
42.	Plumieride	-7.6	-8.1	470.4	-1.54	5	12	0.58
43.	3'-Methoxyfukiic acid	-6.4	-6.5	286.23	-1.43	5	8	-0.03
44.	Methyl 4,6-di-O-galloyl-beta-D-glucopyranoside	-8.7	-8.2	498.4	-0.18	8	14	1.1
45.	Triflusulfuron-methyl	-9.3	-8.9	492.4	3.59	2	9	0.73
46.	L-Homocystine	-4.8	-5.3	268.4	-4.7	6	8	-0.79
47.	3-propylmalic acid	-4.7	-4.9	176.17	-0.55	3	5	-0.47
48.	Ohioensin-A	-8.6	-11.3	372.4	3.92	3	5	-0.40
49.	m-Coumaric acid	-6.1	-6.8	164.16	1.93	2	3	-0.85
50.	Furfural diethyl acetal	-4.5	-5.1	170.21	1.73	0	3	-1.37
51.	7-Hydroxymethyl-12-methylbenz[a]anthracene sulfate	-8.1	-10	352.4	2.95	1	4	-0.76
52.	Streptidine	-7	-6.7	262.27	-3.6	12	6	-0.35
53.	Nifedipine	-6.1	-6.6	346.3	1.51	1	6	-0.09
54.	3-Hydroxy-10'-apo-b,y-carotenal	-7.3	-8.8	392.6	7.43	1	2	-0.33
55.	N-[(5-Hydroxy-2-pyridinyl)methyl]adenosine	-8.6	-8.4	374.35	-0.28	5	9	0.14
56.	9S,12S,13S-trihydroxy-10E-octadecenoic acid	-5.1	-6.7	330.5	3.18	4	5	-0.89
57.	10'-Apo-beta-caroten-10'-al	-7.3	-8.7	376.6	8.52	0	1	0.5
58.	6k-PGF1α-d4	-5.7	-6.6	370.5	2.16	4	6	0.49
59.	9S,11R,15S-trihydroxy-2,3-dinor-13E-prostaenoic acid-cyclo[8S,12R]	-6.2	-6.9	328.4	2.78	4	5	0.86
60.	9,10-Dihydroxy-12,13-epoxyoctadecanoate	-5.6	-6.6	330.5	2.65	3	5	-0.95
61.	Fortimicin KK1	-7.1	-6.8	366.41	-4.57	12	11	-0.01
62.	5-Heptyltetrahydro-2-oxo-3-furancarboxylic acid	-5.9	-6.4	228.28	2.57	1	4	-0.73
63.	Ricinoleic acid	-4.4	-6.2	298.5	5.67	2	3	-0.36

64.	5-Acetoxydihydrotheaespirane	-5.8	-6.5	254.36	3.45	0	3	-1.55
65.	Laserpitin	-6.2	-7.8	450.6	3.36	2	7	0.16

Table No 4. ADMET Profile and Drug likeliness Score –

Sr No.	Ligand Name	HIA	BBB	Acute Oral Toxicity log(1/(mol/kg))	Carcinogenicity
1	4,4-Difluoropregn-5-ene-3,20-dione	0.9903	0.9899	2.811	0.8857
2	Methyl 4,6-di-O-galloyl-beta-D glucopyranoside	0.4490	0.6841	1.956	0.9714
3	Triflusulfuron-methyl	0.9918	0.9706	2.113	0.7143
4	Ohioensin-A	0.9922	0.5263	2.48	0.9857
5	7-Hydroxymethyl-12-methylbenz[a]anthracene sulfate	0.9809	0.9733	1.538	0.5714