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In Silico Pharmacokinetic Properties and Molecular Docking Studies of Phytochemicals from *Abutilon indicum* Leaf Extract and its Effect on Epidermal Growth Factor Receptor as Inhibitor

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Abstract: The main objective of the present study was to perform identification of phytochemicals through GC-MS analysis, Molecular docking and pharmacokinetic (ADME) predictions of selected ligands and standard drugs (Erlotinib and Gefitinib). The study targeted to identify natural biochemical to bind with Epidermal Growth Factor Receptor (EGFR) as anticancer target using molecular docking with the help of standard drugs. Petroleum ether, chloroform, ethanol and aqueous extracts were prepared from the leaves of *Abutilon indicum* for phytochemical analysis. Ethanol and aqueous extracts contained high levels of alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and proteins. The GC-MS analysis identified 131 phytochemicals from the ethanolic leaf extract. The docking score -2486.35 kcal/mol of phytochemical ligand-4 was found to be greater than the docking score -2410.28 kcal/mol of standard drugs. The docking study showed that all four phytochemicals bind to EGFR which confirms that they can fight against cancer. In addition, the ADME predictions reveals the suitability of phytochemical ligand-4 usage in anticancer activity.

Keywords: *Abutilon indicum*, GC-MS Analysis, Molecular Docking, ADME predictions, Druglikeness, Epidermal Growth Factor Receptor

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Introduction

In both developed and emerging economies, carcinoma is one of the most frequent malignancies. Human cancer is a condition in which a few body cells grow rapidly via mitosis and metastasize (Mondal *et al*., 2021). Cancer can develop in any part of the human body. The key attributes of cancer cells include aberrant metabolism, altered nuclear morphology, disrupted cell cycles, repetitive mutations, immune system resilience, systemic inflammation,

invasion creation, and angiogenesis stimulation. Lung carcinoma develops due to abnormal cell proliferation in the lungs. that undergo mitosis rapidly, eventually forming a tumor. Lung cancer is the commonly diagnosed cancer amongst people in terms of epidemiology (Amani *et al*., 2021). Non-small cell lung cancer accounts for 80% of all melanoma within India. The basic concept of molecular precision medicine for lung cancer is the EGFR (Epidermal growth factor receptor), which belongs to the tyrosine kinase receptor family (RTKs) (Sarkar and Ganguly, 2022). EGFR regulates significant cellular processes such as tumor growth, death, and additional cellular processes. EGFR antagonists stop a protein called epidermal growth factor receptor from working (Padmini *et al*., 2020). On tumor tissue, EGFR could be detected at greater levels than usual, leading them to proliferate and multiply. Elevated expression of EGFR could be seen and linked to the etiology of a variety of human cancers (Zhang *et al*., 2020). Thus, targeting the EGFR might control carcinogenesis.

Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness which leads to treatdifferent diseases (Wawrosch and Zotchev, 2021; Sunil *et al*., 2023). According to World Health Organization report, about 80% of the population, mostly in developing countries still depends on traditional medicinal system for their primary health care. India is one of the twelve mega-biodiversity centers with 4 hot spots of biodiversity. Over the last decade there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is a part of time-honored tradition that is respected even today. One of the many plants which are being evaluated for their therapeutic efficacies is *Abutilon indicum* which is commonly known as Thuthi (Tamil). *Abutilon indicum* Linn. (family Malvaceae) is a shrub distributed throughout India (Islam *et al*., 2024). The various parts of the plant (leaves, roots, seeds and seed oil) are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments. The plant contains mucilage, tannins, β-sitosterol, asparagines, flavonoids, alkaloids, hexoses, n-alkane mixtures, alkanol, gallic acid and sesquiterpenes as major phytoconstituents. *Abutilon indicum* has several activities reported as anti-inflammatory, antiproliferative, anti-arthritic, analgesic, sedative, antioxidant, antimicrobial, hepatoprotective, antidiabetic, anticancer, anti-diarrhoeal, anticonvulsant, larvicidal, wound healing, antiasthmatic, diuretic, immuno-modulatory and anti-estrogenic activity (Pavithra *et al*., 2023; Prakash *et al*., 2024; Parasuraman and Ramakrishnan, 2024). The plant is very much used in Siddha medicines. All the parts of plant have medicinal uses. The various secondary metabolites such as phenols, alkaloids, and flavonoids are found in this plant, saponins have an enormous significance in pharmaceutical industry (Raut and Bandawane, 2018). The folk practitioner also uses this plant for curing blood dysentery, fever, allergy and is also an aphrodisiac. The whole plant is used as anti-inflammatory, immune stimulating effect and in piles. It has been reputed in the siddha system of medicine as a remedy for jaundice, piles, ulcer and leprosy (Patel and Rajput, 2013).

In the previous study, medicinal plant namely *Abutilon indicum* was chosen to screen for potential anti-oxidant properties and cytotoxic activity. The extract was also screened to assess the antioxidant activity using 1, 1-Diphenyl-2-picrylhydryzyl [DPPH] radical scavenging activity and Nitric Oxide radical inhibition estimated by the use of Griess reaction with slight modification. These extracts show anti-oxidant properties as well as inhibitory effects on cancer cells with the increased concentration and duration (Saini *et al*., 2015; Bondhare *et al*., 2022).

Phytomedicine is herbal medicine with therapeutic and curative properties. It began with the dawn of human civilisation, and recently researchers have developed therapeutic plants with anti-cancer properties (Balachandran and Govindarajan, 2005). The plant treatment has been proven to prevent or treat many cancers (Lauby-Secretan *et al*., 2015). According to the WHO, non-communicable diseases like cancer account for 63 % of all deaths globally (Singh *et al*., 2012). Plant chemicals have also been recognized as possible anticancer agents (Liu *et al*., 2019). The main objective of the present study is to identify the phytochemicals with anticancer property from the *Abutilon indicum* leaf extract through GC-MS analysis, molecular docking and ADME (Absorption, Distribution, Metabolism and Excretion) prediction studies.

Materials and Methods

Preparation and extraction

Abutilon indicum leaves were collected from the village of Brammadesam, Vembakkam, Thiruvannamalai District, Tamil Nadu, (12°50'26.0"N 79°31'00.0"E; 12.840556 Latitude and 79.516667 Longitude) and then authenticated by Dr. S. Poompozil, Department of Botony, Pachaiyappa's Women's College, University of Madras, Kanchipuram, India. 10 g of the shade dried plant leaves were crushed and extracted in Soxhlet apparatus with 150 ml of petroleum ether, chloroform, ethanol and water. Each solvent was allowed to evaporate, the extracts were stored at 4^{o}C temperature in an airtight container for further examination.

Phytochemical Analysis

With the help of previously established procedure, the leaf extract was tested for phytochemicals (Harborne, 1980; Karthiswaran, 2010). To determine the chemical component profiles of the extracts, a variety of qualitative analytical procedures were performed. The substances that are tested the most frequently are alkaloids, terpenoids, flavonoids, saponins, phenol, tannins, cardiac glycosides, amino acids, proteins, carbohydrates and reducing sugars.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Shimadzu-GCMS-QP-2010 plus (autosystem XL) with ethanolic extract at Nanotechnology Research Centre (NRC), SRM Institute of Science and Technology, Kattankulathur, Chennai, India. Gas chromatograph equipped and coupled to a mass detector SHIMADZURts-5MS5.1 spectrometer, column dimension was 30 meter×0.50 mm ID×1 µm of capillary column. The instrument was set to on initial temperature of 120°C and maintained at this temperature for 0-5 min for solvent delay. At the end of this period, the oven temperature was rose up to 270°C at the rate of an increase of 5°C/min and the analysis completed in 30 min. Injection port temperature was ensured as 260°C and Helium gas flow rate is fixed as 1 ml/min. The ion source temperature in the instrument was fixed as 270°C. The samples were injected in split mode in the ratio of 10:1. Mass spectral scan range was set at 45-450 (m/z). The National Institute of Standards and Technology (NIST) library is used to identify the compounds present in the sample extract. The mass spectrum of individual compound is matched with the mass spectrum of compounds in the sample chromatogram showed as peaks and identified the nature of compounds (Prabha and Bhuvana, 2022).

Molecular Docking studies

The 3D X-ray crystal structure of Epidermal Growth Factor Receptor served as the basis for this investigation (PDB ID: 5wb7). The protein was prepared and docked. The entire computational study was performed on a SwissDock docking server (http://old.swissdock.ch/).

Protein target preparation

The three-dimensional structure of Epidermal Growth Factor Receptor with PDB ID: 5WB7 was downloaded from RCSB Protein Data Bank (PDB) (RCSB Protein Data Bank, 2021) in the .pdb format. After downloading the protein, BIOVIA

Discovery studio visualizer software was used to delete the non-amino acid residues to make the receptor free of any ligands before docking (Biovia *et al*., 2000). The protein preparation process involves addition of hydrogens, removal of water molecules, addition of missing loops, missing atoms in a residue, capping, correcting bond length and bond order, generation of ionization and protonation states, optimization of the hydrogen bond network, removing atomic clashes, and minimization of protein (Polamreddy *et al*., 2018).

Ligand selection and preparation

A total of 131 phytochemicals from *Abutilon indicum*was identified through GC-MS analysis of ethanolic leaf extract. Out of 131 phytochemicals, four compounds selected for *in silico* studies based on the GC-MS area value greater than 3%. The 3D structure of three compounds was downloaded in .sdf format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Kim *et al*., 2021). The 3D structure of one phytocompounds was generated using cactus translate online tool (https://cactus.nci.nih.gov/translate/) (Sitzmann *et al*., 2008) with SMILES and exported in .pdb format for docking studies. The 3D structure of standard drug Erlotinib and Gefitinib were downloaded in .pdb format from drug bank database (https://go.drugbank.com) (Wishart *et al*., 2018) for docking studies. All the compounds were then imported to BIOVIA Discovery studio visualizer and prepare ligands protocol was applied in order to add missing hydrogen bonds and energy minimization using CHARM force fields.

Molecular Docking

Docking of selected four phytochemicals and standard drugs (Erlotinib and Gefitinib) to the EGFR crystal structure (PDB ID: 5WB7) was carried out using the SwissDock Server (Grosdidier *et al*., 2011). The first solution from the docking results considered for the present studies. The docking energy values were utilized for the selection of best docking ligand.

Protein Ligand Interaction

Specific intermolecular interactions between the best ligand docking poses and the binding site of EGFR crystal structure (PDB ID: 5WB7) were further visualised by BIOVIA Discovery studio visualizer software. The docking interactions with important amino acids were analyzed.

Druglikeness (*ADME*) *prediction*

Drug likeliness studies are performed to fulfil a set of guidelines that suggest whether the ligand can be considered as a drug-like compound, thus improving its chances of passing further clinical trials (Chen Yang, 2020). SwissADME (Daina *et al*., 2017) is an online tool developed by EBI and Chem Axon, whose calculations are based on the various drug-likeness conditions: Lipinski's rule (Lipinski, 2004), Veber rule, Ghose filter, Egan filter, and Muegge filter. The six compounds are provided in SMILES format one after the other and the input files submitted for prediction. Consequently, the output file is downloaded as a .csv file.

Results and Discussion

Qualitative phytochemical analysis

The chemical constituents of *Abutilon indicum's* leaf have a bioactive value because they have a strong physiological effect on cells that can develop cancer. The most important of these compounds are phenols, alkaloids, terpenoids, flavonoids and steroids. The qualitative phytochemical analysis of leaf extracts from *Abutilon indicum* leaves is shown in Table 1.

GC-MS analysis of Abutilon indicum

The functional groups that make up the bioactive elements of terpenoids, steroids, fatty acids, phenolic compounds, alkaloids, saponins, and flavonoids can be found using GC-MS analysis (Jeysiha and Palanisamy, 2023). The results of GC-MS analysis of ethanolic leaf extract of *Abutilon indicum* are depicted in Figure 1 and the compounds identified are listed in Table 2.

Table 1: Preliminary phytochemical screening of leaf extract of *Abutilon indicum*

Sl. No	Phytochemicals	Petroleum ether	Chloroform	Ethanol	Aqueous
1.	Alkaloids	-	-	+	-
2.	Terpenoids	-	-	-	-
3.	Flavonoids	+	-	+	-
4.	Saponins	+	+	+	+
5.	Phenols	-	+	+	+
6.	Tannins	-	-	+	+
7.	Cardiac glycosides	-	-	+	-
8.	Amino acids	+	-	+	+
9.	Proteins	-	-	+	+
10.	Carbohydrates	-	+	+	+
11.	Reducing sugars	-	-	+	+

+= Indicated Presence of a phytochemical; - = Indicated Absence of a phytochemical.

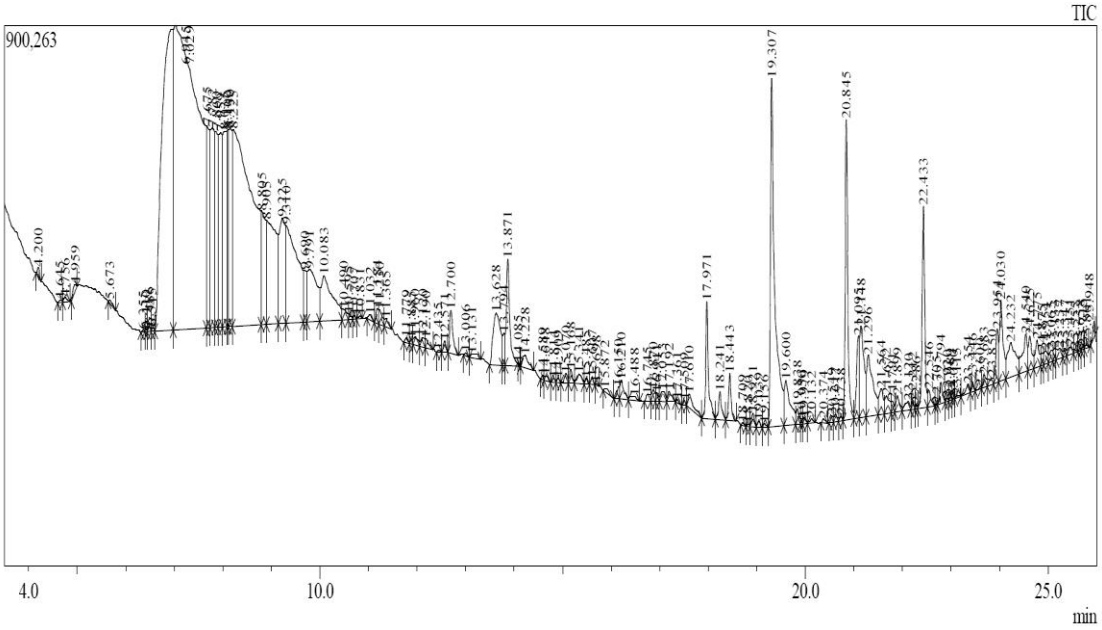


Fig. 1: GC-MS Analysis of ethanolic extract of *Abutilon indicum*.

Molecular Docking studies

Two standard drugs (Erlotinib and Gefitinib) and four ligands (3-(dimethylamino) 1-Propanol, Methane diamine Tetramethyl, 2-Amino butanoic acid, and n-Hexadecanoic acid) selected for the docking studies. These natural phytochemicals active molecules can be screened to pave the way for improving drugs against cancer. All the four phytochemicals, along with standard drugs with dock score were listed in Table 3.

The interacting amino acid residues of the four phytochemicals (ligands) and standard drugs with the target protein is depicted in Table 4.

Drug likeness prediction results

Early on in the drug discovery process, it is

Table 2: GC-MS Analysis of ethanolic leaf extract of *Abutilon indicum* with chemical constituents

Peak	R.Time	Area%	Name
1	4.200	0.06	1-diethoxy-2-methyl- Propane
2	4.645	0.02	1-chloro-3-methoxy-2-Propanol
3	4.756	0.05	1-(1-adamantyl)-3-(dimethylamino)-1-propanone
4	4.959	0.08	2-amino-1-butanol
5	5.673	0.03	1,4-dimethoxy- Butane
6	6.355	0.01	(1e)-2-propenaldimethylhydrazone
7	6.410	0.01	2-methyl-2,4-Pentanediamine
8	6.486	0.04	1-Piperidinepropanoicacid
9	6.545	0.03	Dimethylthexylsilylchloride
10	6.945	10.21	3-(dimethylamino)- 1-Propanol
11	7.029	22.53	Tetramethyl- Methanediamine
12	7.675	2.08	Tetramethyl-2-butyne-1,4-diamine
13	7.782	2.45	4-methyl-1,3-Dioxolane
14	7.860	1.65	Monoethylmalonatemonoamide
15	7.959	2.26	Acetamidinehydrochloride
16	8.035	2.54	1,4-dioxan-2-one
17	8.100	0.77	Dimethyl-2-phosphinoethyl- Amin
18	8.150	1.92	dimethyl-2-phosphinoethyl- Amine
19	8.225	11.72	2-amino- Butanoicacid
20	8.805	1.48	Ethyltrimethyl- Silane
21	8.905	3.14	1,2,2-d3-cyclopentane-1,3-trans-diol
22	9.225	1.99	5-dihydroxy-6-methyl-4H-Pyran-4-one,2,3-dihydro-3
23	9.310	3.40	Trans-3-deuterocyclopentene-3,5-diol
24	9.690	0.50	3,4-dihydro-3-thioxo-1,2,4-Triazin-5(2H)-one
25	9.791	1.53	1-{Bicyclo[2.2.1]heptan-2-yl}ethanone
26	10.083	1.64	Dianhydromannitol
27	10.490	0.15	1,1-Bis[aziridyltrimethylamine]
28	10.595	0.04	Tris(trideuteromethyl)derivativeofcarponiumchlori
29	10.707	0.02	Ephedrine
30	10.831	0.02	3-(dimethylamino)- Propanenitrile
31	11.032	0.05	2-chloro-,trans- Cycloheptanol
32	11.184	0.13	Methyl3-dimethylaminopropionate
33	11.230	0.16	1,1'-oxybis-,(E,E)- 1-Propene
34	11.365	0.09	4-Amino-1-hexanol
35	11.779	0.04	Threo-sec-butyl-3-dn-phenylcarbamate
36	11.885	0.04	1-(2-furyl)-5-dimethylamino- Pent-1-en-3-one
37	11.960	0.11	3,3-dimethyl-4-dimethylamino-2-phenyl-2-butanol
38	12.120	0.12	Dimethoxy-n,n-dimethylmethanamine
39	12.190	0.09	Alanine,phenyl-,methylester
40	12.435	0.02	Di-n-propyl methylamine
41	12.571	0.09	Z,z,z-1,4,6,9-nonadecatetraene
42	12.700	0.43	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobutane
43	13.006	0.02	1-Dichloromethyl(dimethyl)silyloxybutane
44	13.131	0.09	2-[(2-hydroxy-2-methyl-4-phenyl-3-butynyl)amino]butano
45	13.628	1.18	1,3:2,5-Dimethylene-l-rhamnitol
46	13.794	0.22	3,6-Nonadien-1-ol,(E,E)-,TMSderivative
47	13.871	1.16	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one
48	14.085	0.00	Pterin-6-carboxylicacid
49	14.228	0.21	1,6-anhydro- Beta-d-glucopyranose
50	14.589	0.03	3,3-Dimethylbutan-1,2-dioneoxime
51	14.648	0.05	2-cyano-N-(2-phenoxyethyl)- Acetamide

52	14.814	0.03	Glutaricacid,3-methylbut-2-en-1-ylgeranylester
53	14.909	0.03	2,3-epoxy-3,4-dimethyl-,ethylester,cis- Valericacid
54	15.070	0.09	7-Dehydrocholesterylisocaproate
55	15.168	0.12	Ironiodidecomplexi
56	15.341	0.14	Phthalofyne
57	15.485	0.04	1,5,6-trimethyl-2,3,3a,4,7,7a-hexahydro-1h-indene
58	15.607	0.05	2,5-Octadecadiynoicacid,methylester
59	15.698	0.01	Trans-Chrysanthemal
60	15.872	0.06	N-methyl-2-acetoxypropanamide
61	16.150	0.08	(2-Methyl-cyclohex-2-enylidene)-acetaldehyde
62	16.210	0.22	Methylester,(z,z,z)- 8,11,14-eicosatrienoicacid
63	16.488	0.08	1,4,6,6-tetramethyl-1-cyclohexene
64	16.745	0.08	1-fluoro- Tetradecane
65	16.870	0.12	2,3-dimethylcyclohexanol2
66	16.941	0.07	.Beta.,.beta.-galactonicphenylhydrazide
67	17.053	0.16	Isopulegol
68	17.192	0.12	Octadecanoicacid
69	17.361	0.02	Cis-1,2-cyclohexane-1,2,3,3-d4-diol
70	17.509	0.03	3,7-dimethyl- Nonane
71	17.610	0.21	1-methyl-4-(2-methyloxiranyl)- 7-Oxabicyclo[4.1.0]heptane
72	17.971	1.01	Neophytadiene
73	18.241	0.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
74	18.443	0.37	Neophytadiene
75	18.709	0.02	1,6-dideoxydulcitol
76	18.820	0.02	3,4-dihydroxyproline
77	18.921	0.13	13,16-Octadecadiynoicacid,methylester
78	19.039	0.05	12-Methyl-E,E-2,13-octadecadien-1-ol
79	19.156	0.03	1,2-epoxy- Decane
80	19.307	4.73	N-Hexadecanoicacid
81	19.600	0.83	Heptadecanoicacid,ethylester
82	19.838	0.14	1-phenyl-1-bromo-2,2-dimethylpropane
83	19.930	0.03	Ethyl 3-(diethylamino)propanoate
84	19.979	0.05	Ethanimidothioicacid,2-(dimethylamino)-n-[(methylami
85	20.132	0.05	3-Oxatricyclo[3.2.1.0(2,4)]octane
86	20.374	0.04	3-o-hexopyranosylhex-2-ulofuranosylhexopyranoside
87	20.549	0.05	1-methoxy- Dodecane
88	20.617	0.07	3-bis[cycloazapropyl]- 2,4-Diazapentane,N,N'-dimethyl-3
89	20.738	0.04	4-cycloocten-1-ylethylether
90	20.845	2.42	3,7,11,15-tetramethyl-2-hexadecen-1-ol
91	21.095	0.67	9,12-Octadecadienoicacid
92	21.148	1.26	9,12,15-Octadecatrien-1-ol
93	21.296	1.20	9-octadecenoicacid(z)
94	21.564	0.35	Octacosanoicacid,2,4,6,8-tetramethyl-,methylester
95	21.672	0.26	Phenanthrene,7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-te
96	21.791	0.10	2-hexadecen-1-ol,3,7,11,15-tetramethyl-,[r- [r*, r*-(e)]]-
97	21.899	0.19	3,7-dimethyl-6-octenal
98	22.120	0.14	Octadecanoicacid,2,3-dihydroxypropylester
99	22.212	0.07	Trimethylsilyl-di(timethylsiloxy)-silane
100	22.286	0.03	Cyclopentolate
101	22.433	1.64	3-Cyclopentylpropionicacid,2-dimethylaminoethylester
102	22.546	0.16	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione
103	22.705	0.01	1-propanone,1-cyclopropyl-, o-methyloxime
104	22.794	0.20	Hexadecyl- Oxirane
105	22.920	0.02	Aceticacidtricyclo[4.2.1.02,5]non-7-en-3-yl ester
106	22.989	0.02	Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11-dimethyl
107	23.050	0.00	2-(dimethylamino) ethyl1-adamantanecarboxylate

108	23.115	0.02	Octanoicacid,2-chloroethylester
109	23.354	0.10	7-hexadecenal
110	23.446	0.07	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-heptasiloxane
111	23.600	0.02	1-[2-Deoxy-.beta.-d-erythro-pentofuranosyl]pyrrole-2,4-dicarboxamide
112	23.683	0.13	Pentadecanal
113	23.850	0.10	Trans-3'-Hydroxycotinine,trimethylsilylether
114	23.954	0.46	1,5-benzothiazepin-4(5h)-one,3-(acetyloxy)-5-[2-(dimethyl
115	24.030	0.87	1,5-benzothiazepin-4(5h)-one,3-(acetyloxy)-5-[2-(dimethyl
116	24.232	0.88	Nonadecanoicacid,TMSderivative
117	24.540	0.56	Docosanal
118	24.625	0.35	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyloctasiloxa
119	24.775	0.32	Diisooctylphthalate
120	24.875	0.10	2-(9-Fluoro-[1,2,4]triazolo[4,3-c]quinazolin-3-yl)pyrazine
121	24.954	0.14	Methyl10-oxooctadecanoate
122	25.065	0.13	BisabololoxideB
123	25.133	0.10	4-(2,2-dimethyl-6-methylenecyclohexyl)- 2-Butanone
124	25.237	0.16	2-[[(6-nitro-1,3-benzodioxol-5-yl)methylene]amino]- Ethanol
125	25.347	0.07	4-methoxy-1-nitro-2-(trifluoromethyl)- Benzene
126	25.451	0.11	Ethyl2-[3-(pyrazin-2-yl)-1H-1,2,4-triazol-5-yl]acetate
127	25.555	0.02	Nonanoicacid,6-chloro-,chloromethylester
128	25.658	0.01	Methyl4-bromo-2,2-dimethyl-3-(nitromethyl)butanoate
129	25.715	0.02	2-(hydroxymethyl)-2-{[(4-nitrophenyl)methylidene]amin
130	25.801	0.03	3-[4-Azido-5-imidazolyl]propenoicacid,methylester
131	25.948	0.09	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl

Table 3: The docked complexes along with energy values

S. No.	EGFR docked Complex	Full fitness Energy value(Kcal/mol)
1.	1-Propanol, 3-(dimethylamino) (Ligand 1)	-2449.93
2.	Methanediamine, Tetramethtl(Ligand 2)	-2451.49
3.	Amino, butanoic acid (Ligand 3)	-2422.12
4.	n-Hexadecanoic acid (Ligand 4)	-2486.35
5.	Erlotinib (Drug 1)	-2410.28
6.	Gefitinib (Drug 2)	-2410.28

Table 4: The interacting amino acids in the binding pockets

S. No	Complex	Amino acids
1.	Ligand 1	Lys 303; Lys 304; Glu 306
2.	Ligand 2	Glu 306; Lys 303; Lys 304
3.	Ligand 3	Met 294; Lys 303; Tyr 292; Pro 308
4.	Ligand 4	Pro 242; Met 244; Leu 243; Cys 240
5.	Drug 1	Ser 11; His 409; Asn 12; Thr 406; Gly 410; Arg 29
6.	Drug 2	Met 253; Leu 243; Pro 242; Lys 260; Cys 240; His 280; Asp 279

important to investigate the pharmacokinetic characteristics of potential therapeutic candidates. Molecular weight (MW)< 500 Da, number of hydrogen bond donors (HBD's)< 5, number of hydrogen bond acceptors (HBAs)< 10, and octanol-water partition coefficient (LogP)< 5 are the requirements for drug-like compounds, according to Lipinski and his team – Rule of 5 (RO5). There should not be more than one violation (Singh *et al*., 2017). The bioavailability radar quickly determines the significant physicochemical characteristics and drug-likeness

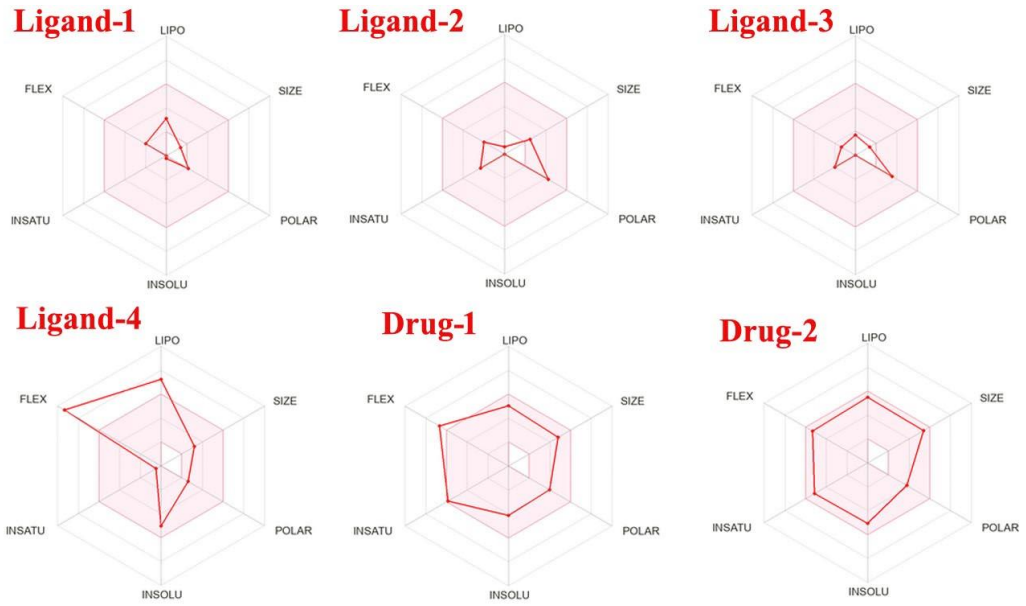


Fig. 2: Bioavailability radar image of ligands and drugs.

Table 5: Druglikeness predictions of four ligands and two standard drugs

S. No.	Phytochemicals	Lipinski Rule	Ghose Rule	Veber Filter	Egan Filter	Muegge Filter	BioScore
	1-Propanol, 3-(dimethylamino)-	Yes; 0 violations	No; 2 violations	Yes	Yes	No; 1 violation	0.55
	Methanediamine, Tetramethtl	Yes; 0 violations	No; 0 violations	Yes	Yes	No; 2 violations	0.55
	2- Amino, butanoic acid	Yes; 0 violations	No; 3 violations	Yes	Yes	No; 3 violations	0.55
	n-Hexadecanoic acid	Yes; 1 violations	Yes	No;1 violation	Yes	No; 1 violation	0.85
	Erlotinib	Yes; 0 violations	Yes	Yes	Yes	Yes	0.55
	Gefitinib	Yes; 0 violations	Yes	Yes	Yes	Yes	0.55

of the selected compounds and standards (Daina and Zoete, 2016). The bioavailability radar is depicted in Figure 2. Druglikeness predictions of four ligands and two standard drugs are listed in Table 5.

The SwissADME online server was used to calculate the ADME values (absorption, distribution, metabolism, excretion) and druglikeness is displayed in Table 6. Since high-quality drug candidates must have both sufficient efficacies against the therapeutic target and suitable ADME properties at a therapeutic dose, ADME qualities are crucial at the beginning of drug discovery and development (Guan *et al.*,

Table 6: Pharmacokinetic profile and druglikeness prediction of leaf extract of *Abutilon indicum*

Molecule	Ligand-1	Ligand-2	Ligand-3	Ligand-4	Drug-1	Drug-2
Formula	C5H13NO	C7H16N2O4	C4H9NO2	C16H32O2	C22H23N3O4	C22H24ClFN4O3
MW	103.16	192.21	103.12	256.42	393.44	446.9
Heavy atoms	7	13	7	18	29	31
Aromatic heavy atoms	0	0	0	0	16	16
Fraction Csp3	1	0.71	0.75	0.94	0.27	0.36
Rotatable bonds	3	3	2	14	10	8
H-bond acceptors	2	6	3	2	6	7
H-bond donors	1	2	2	1	1	1
MR	30.21	47.21	25.82	80.8	111.4	121.66
TPSA	23.47	81.08	63.32	37.3	74.73	68.74
iLOGP	1.88	0.96	0.8	3.85	3.67	4.04
XLOGP3	-0.06	-4.41	-2.53	7.17	3.31	4.11
WLOGP	-0.07	-0.78	-0.19	5.55	3.48	4.32
MLOGP	0.23	-0.64	-2.61	4.19	1.89	2.82
Silicos-IT Log P	-0.25	-0.86	-0.73	5.25	4.06	4.31
Consensus Log P	0.35	-1.15	-1.05	5.2	3.28	3.92
ESOL Log S	-0.24	1.94	1.25	-5.02	-4.11	-5.05
ESOL Solubility (mg/ml)	58.8	16900	1820	0.00243	0.0303	0.00395
ESOL Solubility (mol/l)	0.57	88	17.6	0.00000949	0.0000771	0.00000883
ESOL Class	Very soluble	Highly soluble	Highly soluble	Moderately soluble	Moderately soluble	Moderately soluble
Ali Log S	0.02	3.32	1.74	-7.77	-4.56	-5.26
Ali Solubility (mg/ml)	108	404000	5730	0.00000431	0.011	0.00246
Ali Solubility (mol/l)	1.04	2100	55.5	0.0000000168	0.0000278	0.0000055
Ali Class	Highly soluble	Highly soluble	Highly soluble	Poorly soluble	Moderately soluble	Moderately soluble
Silicos-IT LogSw	-0.56	-0.43	0.34	-5.31	-7.26	-7.94
Silicos-IT Solubility (mg/ml)	28.7	71.2	225	0.00125	0.0000215	0.00000514
Silicos-IT Solubility (mol/l)	0.278	0.371	2.18	0.00000488	0.0000000546	0.0000000115
Silicos-IT class	Soluble	Soluble	Soluble	Moderately soluble	Poorly soluble	Poorly soluble
GI absorption	High	High	High	High	High	High
BBB permeant	No	No	No	Yes	Yes	Yes
Pgp substrate	No	No	No	No	No	No
CYP1A2 inhibitor	No	No	No	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No	Yes	Yes
CYP2C9 inhibitor	No	No	No	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	Yes	Yes
CYP3A4 inhibitor	No	No	No	No	Yes	Yes
log Kp (cm/s)	-6.97	-10.6	-8.73	-2.77	-6.35	-6.11

Lipinski violations	0	0	0	1	0	0
Ghose violations	2	1	3	0	0	0
Veber violations	0	0	0	1	0	0
Egan violations	0	0	0	0	0	0
Muegge violations	1	2	3	1	0	0
Bioavailability Score	0.55	0.55	0.55	0.85	0.55	0.55
PAINS alerts	0	0	0	0	0	0
Brenk alerts	0	2	0	0	1	0
Leadlikeness violations	1	1	1	2	2	3
Synthetic Accessibility	1	1.61	1.1	2.31	3.19	3.26

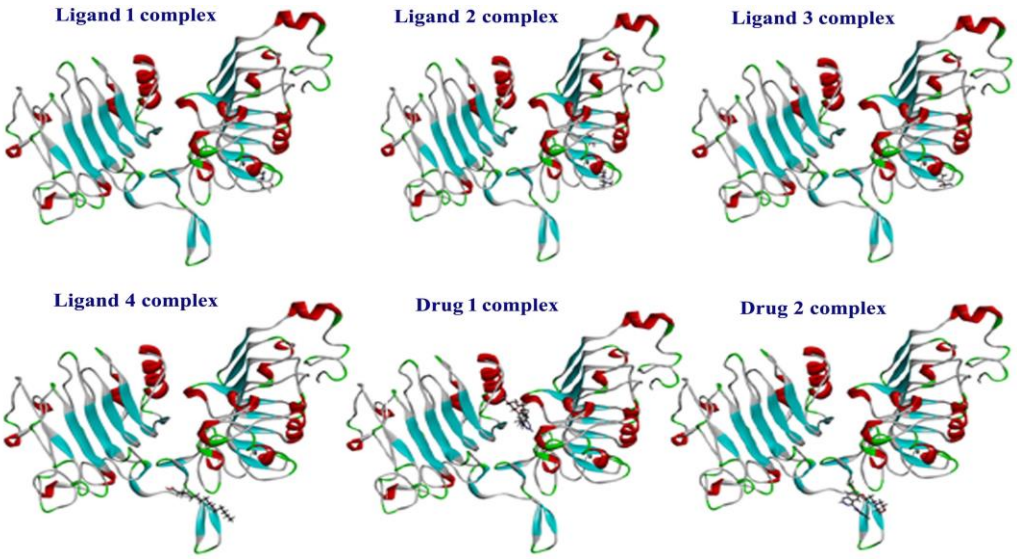


Fig. 3: Docked EGFR complex diagrams with four ligands and two standard drugs.

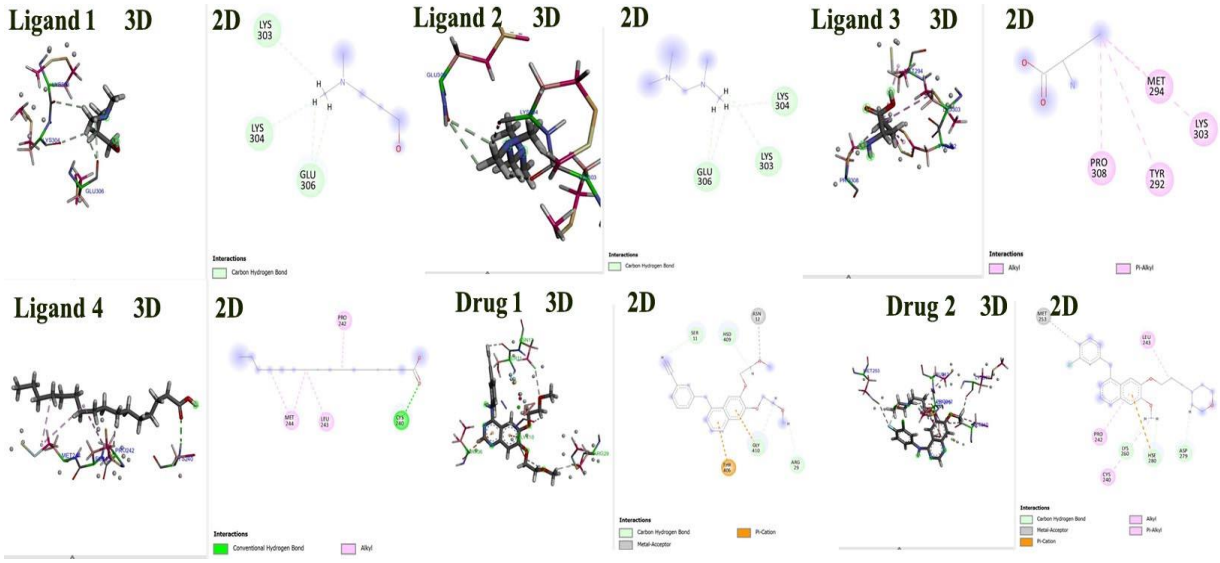


Fig. 4: 3D and 2D interaction images between EGFR with four ligands and two standard drugs.

2018).

As can be seen in Table 6, ligand-4 compound's HA, MW, HBD, HBA, and WLog P values fall within the allowed range specified in the RO5, and ligand-4 compound not violated more than one rule, in contrast to the other ligands (phytochemicals), which have more than one violation.

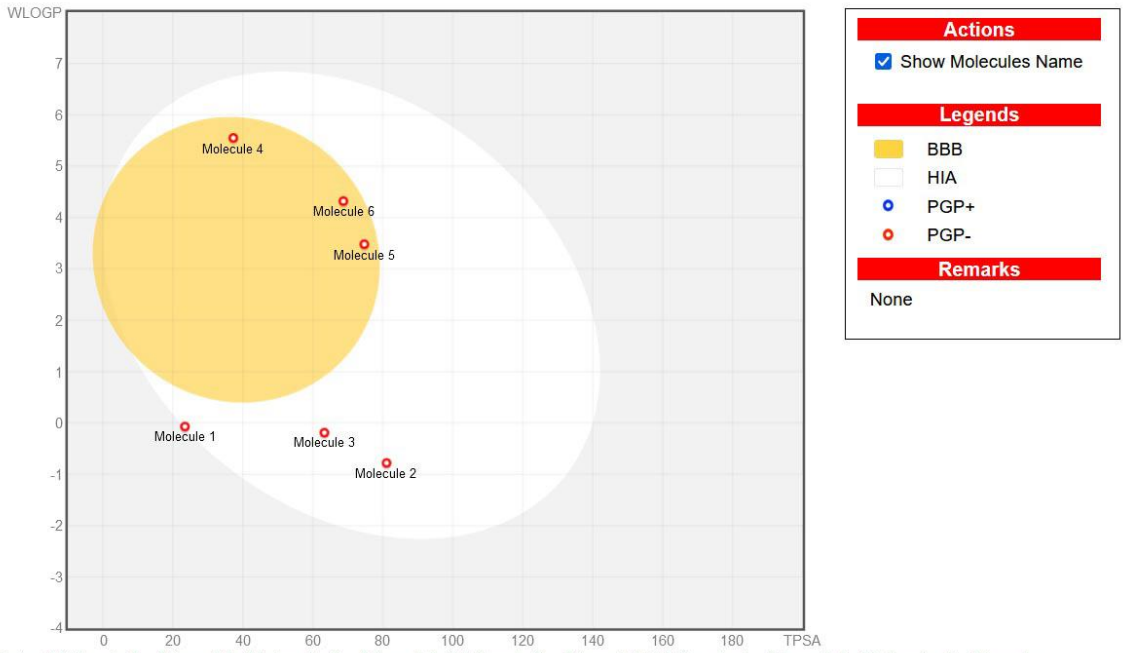
The result of the present study indicated that the *Abutilon indicum* have important phytochemical constituents. It was evident that ethanolic extract of *Abutilon indicum* found to contain high levels of alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and proteins. Alkaloids, terpenoids, flavonoids, and glycosides were absent in aqueous extract of leaf of *Abutilon indicum*. Terpenoids was absent in ethanolic extract of *Abutilon indicum*. Many of the commonly available and medicinally important phytochemicals that were found in a spectrum of medicinal plants are used in preventing and treating a wide variety of diseases, disorders and infections (Nithyatharani and Kavitha, 2018). Based on the qualitative phytochemical analysis, it was concluded that ethanol is a best solvent for the extraction purpose.

GC-MS analysis report showed the presence of 131 active principles in leaf extract of *Abutilon indicum* . Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics (Sekar and Saranya, 2016). Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but present studies showed that ethanolic extract of the plant was certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent. In the present study, ethanolic extract of *Abutilon indicum* revealed the presence of 131 active chemical constituents. These compounds having Antimicrobial, Antifouling, Antioxidant, Anti-inflammatory and Anticancer properties (Prabha and Bhuvana, 2022).

The three-dimensional structure of Epidermal Growth Factor Receptor (PDB ID: 5WB7) was prepared for docking using BIOVIA software (Sarkar and Ganguly, 2022). 3-(dimethylamino) 1-Propanol, Methanediamine Tetramethyl, 2-Amino butanoic acid, and n-Hexadecanoic acid were selected as ligands from the 131 active chemical constituentsbased on the GC-MS area value greater than 3%. Docked structures are shown in Figure 3.

The phytocompound n-Hexadecanoic acid (ligand-4) showed very good binding affinity (-486.35 kcal/mol) with the amino acid residues Pro 242; Met 244; Leu 243; and Cys 240 of target protein. The lowest binding affinity of -2422.12 kcal/mol was observed between the phytocompound 2-Amino, Butanoic acid (ligand-3) and the amino acid residues such as Met 294; Lys 303; Tyr 292; and Pro 308 of target protein.The binding affinity of -2410.28 kcal/mol was reported for the Erlotinib (drug 1) and the target protein with the amino acid residues of Ser 11; His 409; Asn 12; Thr 406; Gly 410; and Arg 29. The binding affinity of -2410.28 kcal/mol was reported for the Gefitinib (drug 2) and the target protein with the amino acid residues of Met 253; Leu 243; Pro 242; Lys 260; Cys 240; His 280; and Asp 279. From the docked energy value, it was identified that ligand 4, was found to be the good lead compound and it might be utilized for the drug development process (Saravanan *et al*., 2021).

The 2D and 3D interaction of the four phytocompounds (ligands) and two standard drugs with the target protein is depicted in



Note: Molecule 1 – Ligand 1; Molecule 2 – Ligand 2; Molecule 3 – Ligand 3; Molecule 4 – Ligand 4; Molecule 5 – Drug 1; Molecule 6 – Drug 2.

Fig. 5: Boiled egg diagram of four ligands and two standard drugs.

Figure 4. Drug 2 and ligand 4 were found to have the same binding pockets. So, they might exert same effect of anticancer property. Druglikeness studies are performed to fulfil a set of guidelines that suggest whether the ligand can be considered as a drug-like compound, thus improving its chances of passing further clinical trials (Chen Yang, 2020). ADME physicochemical and pharmacokinetic properties were tested for the four selected phytocompounds of the ethanolic leaf extract of *Abutilon indicum* and two drugs Erlotinib and Gefitinib. Lipophilicity ($XLogP_3$), topological polar surface area (TPSA), solubility and hydrophobicity (Log S), carbon fraction sp3 (saturation carbons in sp3 hybridization) and rotatable bonds (flexibility), Lipinski rule, blood–brain barrier (BBB), human intestinal absorption (HIA), P-glycoprotein (PGP), cytochrome P450 inhibitor isoenzymes and skin permeation parameters were evaluated for all the compounds. The phyto-compounds that best interacted obey Egan filter, as well as the synthetic drugs. Most of the compounds cross BBB and had high intestinal absorption. A bioavailability score of 0.55 was observed in the phytocompounds ligand-1, ligand-2, ligand-3 and drugs, and but for ligand-4, it was 0.85 (Saravanan *et al*., 2021). By considering the violations and bioavailability score, the ligand 4 considered as best lead with minimal violation. From the boiled egg diagram (Fig. 5) and druglikeness predictions, ligand 4 was found to have less violations and docking score. Thus, ligand 4 i.e. n-Hexadecanoic acid will be selected as lead for further studies.

Conclusion

Many of the commonly available and medicinally important phytochemicals that were found in plants that are used in preventing and treating a wide variety of diseases, disorders and infections. It is seen from the literature that *Abutilon indicum* is a very important plant for its large number of medicinal properties as well as medicinally important chemicals like flavonoids, tannins, carbohydrates, glycosides, and phenolic compounds.

Through GC-MS studies, 131 active principles in the leaf extract of *Abutilon indicum* were identified in the present study. The result of the present study provided supportive scientific evidence that the leaf extract of *Abutilon indicum* which are pharmacologically active in treatment of some aliments such as cancer. Due to the presence of pharmacologically active phytochemicals in *Abutilon indicum* leaves, they might be used as an alternative for the preparations for medical herbal products. Further studies have to be carried out to identify the bioactivities of the 131 identified compounds from the present study which might be useful for the medicinal applications of the plant *Abutilon indicum*.

The use of docking studies allows to comprehend how lead ligands interact with therapeutic target. Docking is crucial in the field of structure-based drug discovery for identifying new biologically active compounds. According to the molecular docking study and druglikeness predictions, among the selected 4 phytochemicals, ligand 4 was found to have less violations and greater docking score. Concluding *Abutilon indicum* extracts possess numerous secondary metabolites with promising pharmacological applications that has traditionally been utilized as a traditional herbal medicine to treat infection and cancer. Hence, ligand 4 i.e. n-Hexadecanoic acid could be a potential therapeutic option for further consideration ofdrug development to cancer.

Ethical Statement

No animals or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Hindumathi A.: Carried out experiments, data collection, preparation of the original manuscript; *Sivakumar S.*: Experimental planning, supervision, data validation, manuscript editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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