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Research Article

Molecular Docking And Anticancer Activity Of Isolated Compound From *Bruguiera Cylindrica* Leaf Extract.

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Abstract:
In research, the anticancer property of 7-(dimethyl amino)-3ethyl-8-oxo-5-thia-1-azabicyclo (4.2.0)-Oct-2-ene-2-carboxylic acid (LS2) that was purified from *B. cylindrica* has been studied. For evaluating the anticancer efficacy of the compound two cancer cell lines (human breast (MCF-7) and lung cancer cell lines (A549) were chosen for both *in vitro* by MTT assay and *in silico* studies using autodock 4.2 in MCF-7 and A549 in IC_{50} was anticancer activity for the compound had increased with an increase in concentration with IC_{50} was 58.81 µg (MCF7) and 70.8 µg for (A549). Further confirmation studies were done by docking of the compound with the proteins of the above-examined cancer cell lines in compared with the typical drug doxorubicin. According to the docking investigations, LS2 exhibited similar results with higher binding energy, constant inhibition, and a greater number of amino acid interactions involving hydrogen bonds in comparison to the standard drugs.

Keywords: *B. cylindrica*, doxorubicin, MTT-assay, docking, bioactive.

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Introduction:

Phytopharmaceuticals are the bioactive compounds with proven health benefits and mangroves are one of such plant resource with potential therapeutic applications. Since ancient times, mangroves had a prominent role in folk medicine for treating various microbial infections, cardiovascular diseases and cancer because of their unique chemical profile composed of various limonoids, fatty acids, phenolic, alkaloids, lignin, terpenoids and coumarins (Taniguchi *et al.*, 2018). Mangroves are a unique plant community that grows in high environmental stress like estuarial wetlands with high saltiness, high temperature, low nutrients, and extreme radiation produce unique metabolites to combat the stress. The Rhizophora mangle vegetation produces several enzymes and defense compounds, containing polyphenolic compounds that are considered to serve as ultraviolet screening compounds (Adame *et al.*, 2021; Dahibhate *et al.*, 2020). In India, a diversified mangrove forest ecosystem is found coastal states of Kerala, Gujarat, Karnataka, Maharashtra, Goa, TN, Odisha, AP, WB, Pondicherry, Diu and Daman in the Gulf of Mannar. It spreads about 6,749 sq. km along the sea shore, including the Andaman and Nicobar Islands (Ramachandra and Kishore, 2019). Only limited research has been performed on these plants for their anticancer potential. Hence, in this research has concentrated on evaluating the anticancer properties of the leaves of *B. cylindrica* under *in vitro* and *in silico* molecular studies.

Materials and methods:

Source of Compound:

"7-(dimethyl amino)-3ethyl-8-oxo-5-thia-1-azabicyclo (4.2.0)-Oct-2-ene-2-carboxylic acid" bioactive compound was extracted by soxhlet apparatus with five solvents like ethanol, butanol, aqueous, ethyl acetate and methanol. Purified and identified by using standard methods such as FTIR, LC-MS and NMR. The ethyl acetate solvent extraction of *B. cylindrica* leaves was used in this study. (Kumari and Kasturi, 2024). The compound has been coded as LS2 for easy reference purposes.

Cell lines Maintenance:

The National Centre for Cell Science (NCCS) in Pune, India, is where the cell lines MCF7 and A549 were obtained. Breast and lung cancer were the most common types of cancer, as indicated by the expected number of new cases in 2018 (TianJ *et al.,* 2020). The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) (Hi-media) supplemented with 10% FBS (fetal bovine serum) and antibiotics such as penicillin (0.5 ml^{-1}) under a controlled environment consisting of 5% CO2 and 95% air at a temperature of 37°C.

Evaluation of Anticancer activity by MTT assay of LS2:

MTT assay evaluated cell viability with 3 individual experiments with six concentrations of compound in triplicates was performed Cells were trypsinized and the trypan blue assay was conducted to identify viable cells in the suspension of cell. The cells were counted using a haemocytometer and planted at a density of 5.0 x 10^3 cells per well with 100 µl of media in 96-well plate culture medium. The cells were then incubated at 37°C for the whole night. Following incubation, the old media was removed, and 100 µl of new media containing various doses of the test compound was added to the wells. After that, the wells incubated for an additional 24 hours. Following three hours of incubation at 37°C, the drug solution was removed from each well, and a new medium containing MTT solution (0.5 mg/ml^{-1} in phosphate buffer solution) was added. By using cells with metabolically active mitochondria, the MTT salt was broken down into chromophore formazan crystals, which led to the formation of precipitates at the end of the incubation time. The optical densities of solubilized crystals in DMSO were measured at 570 nm on a micro plate reader. The % growth inhibition was estimated through the use of the following formula (Darmadi *et al.,* 2021).

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The IC_{50} value was defined by using a linear regression equation i.e., y =mx+c. At this time,
y= 50, m and c values came from the practicality diagram.

***In silico* studies:**

preparation of Ligand:

The structure of the standard drug doxorubicin was acquired from Drug Bank, and the pure compound LS2, which was isolated, purified, and spectrally characterized from plant extract, was sketched using Marvin sketch program. PyMol® software was utilized to visualize the three-dimensional structures of the compound and drug, which were generated in PDB files (Kim *et al.,* 2015).

Preparation of Protein:

From a protein data bank (PDB), the crystallographic protein structures of cancer cell lines were retrieved. Using auto ligand, the active site of these proteins was identified, and the Molecular Graphics Program, PyMol® software was used for visualized the active site of proteins. (Sanner 1999; Albin and Harris, 2010). The energy was minimized using Swiss PDB version 1.4.2, and the macromolecule was downloaded in PDB format.

Docking analysis:

The interactions between macromolecules and ligands were determined using the auto-dock version 4.2 tools of the Molecular Graphics Laboratory (MGL) and the addition of converting PDB and PDBQT files The grid maps were pre-calculated using 60, 60 and 60 A° (x, y, and z) to contain all the amino acid residues found in the receptor. These maps were constructed using the PDBQT files of the macromolecule and ligand. The Lamarckian genetic algorithm (LGA) was used to identify and evaluate a maximum of 10 conformers required for the docking procedure, using a grid spacing of 0.375A°. The population contained 150 individuals. The rest of the parameters were configured using the default settings of the auto dock. After the docking process, the most suitable postures were examined for hydrogen bonding/π-π interactions and subjected to root mean square (RMS) calculations using PyMol® software. The docking process was completed using a DELL laptop with Windows 10, with an Intel CORE TM i3 processor, a 64-bit operating system, and 4GB of RAM (Liu *et al.,* 2017; Vasanthi *et al.,* 2017; Singla and Jaitak, 2015).

Results:

Percent inhibition by LS2 was increased with increased in the concentration against A549 was 3.98 % in 5 µg, 9.48 % in 10 µg, 21.82 % in 25 µg, 44.02 % in 50 µg, 65.08 in 100 µg and IC_{50} is 70 µg (Table:1). Whereas percent inhibition against MCF7 was 5.69 % in 5µg, 13.41% in 10 µg, 34.92 % in 25 µg, 54.59 % in 50µg, 71.32 in 100 µg and IC_{50} is 58.81 µg (Table:2).

Docking studies:

Retrieved three-dimensional structures of A549 lung cancer cell line (Matrix Metallo Proteinase (PDB id: 1BQO), MCF7 breast cancer cell line (HER2 protein (PDB id: 3PP0) from the protein data bank (PDB) and Doxorubicin standard anticancer drug (Drug bank id: DB00997) from the drug bank and fraction LS2 was sketched by using Marvin Sketch and visualized in PyMol® and presented in (Fig. 1).

Active site determination of proteins of cancer cell lines:

The amino acids that were existing in the active site of protein A549-Lung cancer (Matrix Metallo Proteinase (PDB id- 1BQO) (GLY179, ILE180, LEU181, ALA182, LEU214, THR215, HIS218, GLU219, PRO232, LYS233, ALA234, VAL235, MET236, PHE237, PRO238, THR239, TYR240, LYS241, VAL243, PHE248, ARG249) and MCF7 Breast cancer (HER2 protein (PDB id- 3PP0) (GLY776, GLY778, THR835, VAL777, MET801, THR862, SER783, THR798) were identified by using auto ligand software.

Molecular docking interactions:

To find the optimal docking pose based on the ligands docking score list, total of 10 runs were carried out. The macromolecule and ligand interactions was observed in expression of binding energy, number of H_2 bonds, number of binding sites, and length of H_2 bonds following the completion of docking simulations.

Interaction of Doxorubicin with 1BQO protein of A549 cell line:

The docking simulations of the Doxorubicin ligand with 1BQO produced conformers with a cluster of 2.0 Aº out of 10 docking runs, with a root mean square (RMS) tolerance. The binding energy of the ligand was identified to be the greatest, at 7.37 kcal/mol, while the reference RMS value was determined to be 32.59. The ligand interaction with the two hydrogen bonds at THR220 and SER225 on the protein's active site was observed, with bond lengths of 2.63 Aº and 2.56 Aº, respectively. The THR220 protein functions as a binding site between two amino acids, as seen in (Fig: 2).

Interaction of LS 2 with 1BQO protein of A549 cell line:

The docking simulations of the interaction between pure fraction 3 and 1BQO resulted in conformers with a single cluster of 2.0 Aº out of 10 docking experiments with a root mean square (RMS) tolerance. The ligand had the greatest binding energy of -4.43 kcal/mol, whereas the reference root mean square (RMS) value was 31.68. The ligand interacted with the two hydrogen bonds present at HIS224 and SER225 on the active sites of the protein, with bond lengths of 2.63Ao and 2.68Ao, respectively. The active sites of the protein have been determined to include SER225, with bond lengths of 2.63Ao and 2.68Ao, as seen in (Fig: 2).

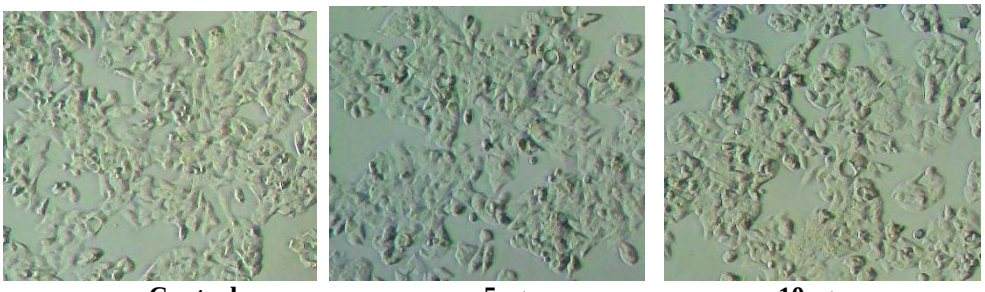
Interaction of Doxorubicin with 3PP0 with 3PP0 protein of MCF-7 cell line:

The docking simulations have shown that the interaction between Doxorubicin and 3PP0 produced conformers with a single cluster of 2.0 Aº out of 10 docking runs, with a root mean square (RMS) tolerance. The binding energy of the ligand was identified to be -9.03 kcal/mol, whereas the reference root mean square (RMS) value was determined to be 31.32. The ligand interacted with the two hydrogen bonds located at LYS753 and ASP850 on the protein's active site, with bond lengths of 2.83 Aº and 2.75 Aº respectively. ASP850 functions as a binding site among the two amino acids, as seen in (Fig: 2).

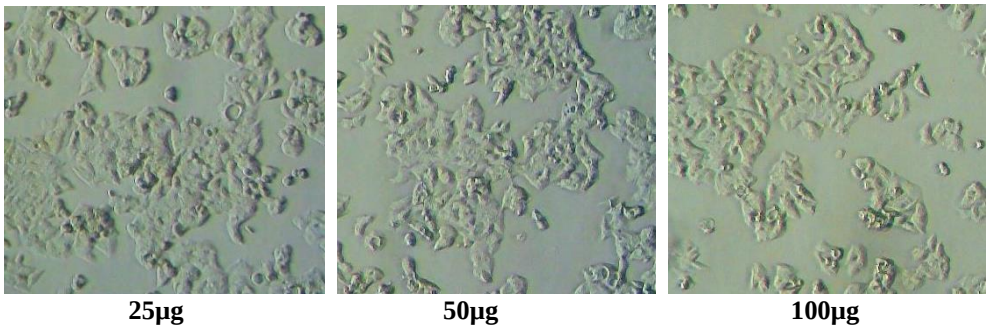
Interaction of LS2 with 3PP0 with 3PP0 protein of MCF-7 cell line:

The docking simulations showed that the interaction between fraction 3 and 3PP0 resulted in conformers with a single cluster of 2.0 Ao out of 10 docking runs, with a root mean square (RMS) tolerance. The binding energy of the ligand was identified to be the highest, -6.29 kcal/mol, whereas the reference root mean square (RMS) value was 32.08. The ligand interacted with a hydrogen bond with the protein's active site at position MET801, with a bond length of 2.71Aº. The binding site of MET801 is shown in (Fig: 2).

A549

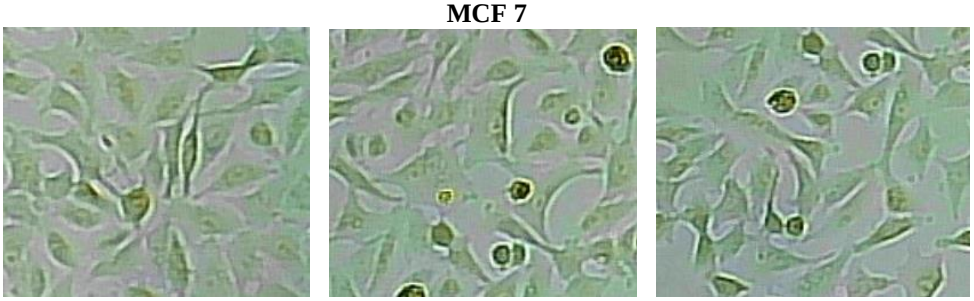


Control **5µg** **10µg**

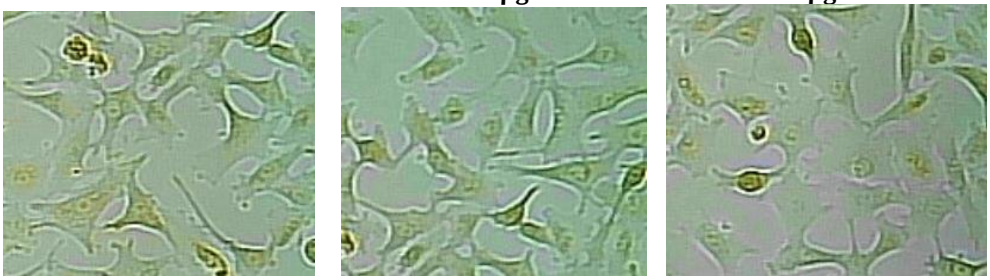


25µg **50µg** **100µg**

MCF 7



Control **5µg** **10µg**



25µg **50µg** **100µg**

Table: 1 anticancer activity of LS2 against MCF-7

Concentration(µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
05	0.513	5.69	94.31	
10	0.471	13.41	86.59	
25	0.354	34.92	65.08	
50	0.247	54.59	45.41	
100	0.156	71.32	28.68	
Untreated	0.544	0	100	
Blank	0	0	0	58.81

Table: 2 anticancer activity of LS2 against A549

Concentration(µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
05	0.506	3.98	96.02	
10	0.477	9.48	90.52	
25	0.412	21.82	78.18	
50	0.295	44.02	55.98	
100	0.184	65.08	34.92	
Untreated	0.527	0	100	
Blank	0	0	0	70.8

Molecular docking analysis:

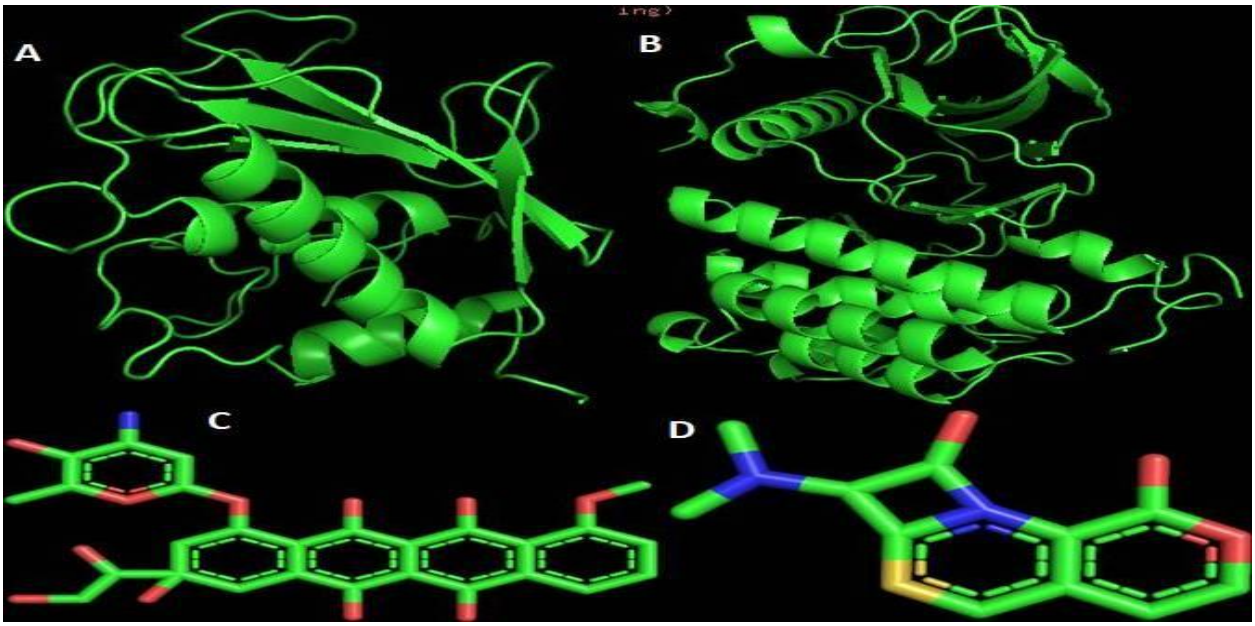


Fig: 1 3D structures of ligands and proteins

(A) Lung cancer-A549 (Matrix Metallo Proteinase (PDB id- 1BQO); (B) Breast cancer -MCF7 (HER2 protein (PDB id- 3PP0); (C) Standard drug-Doxorubicin (Drug bank id- DB00997); (D) LS 2.

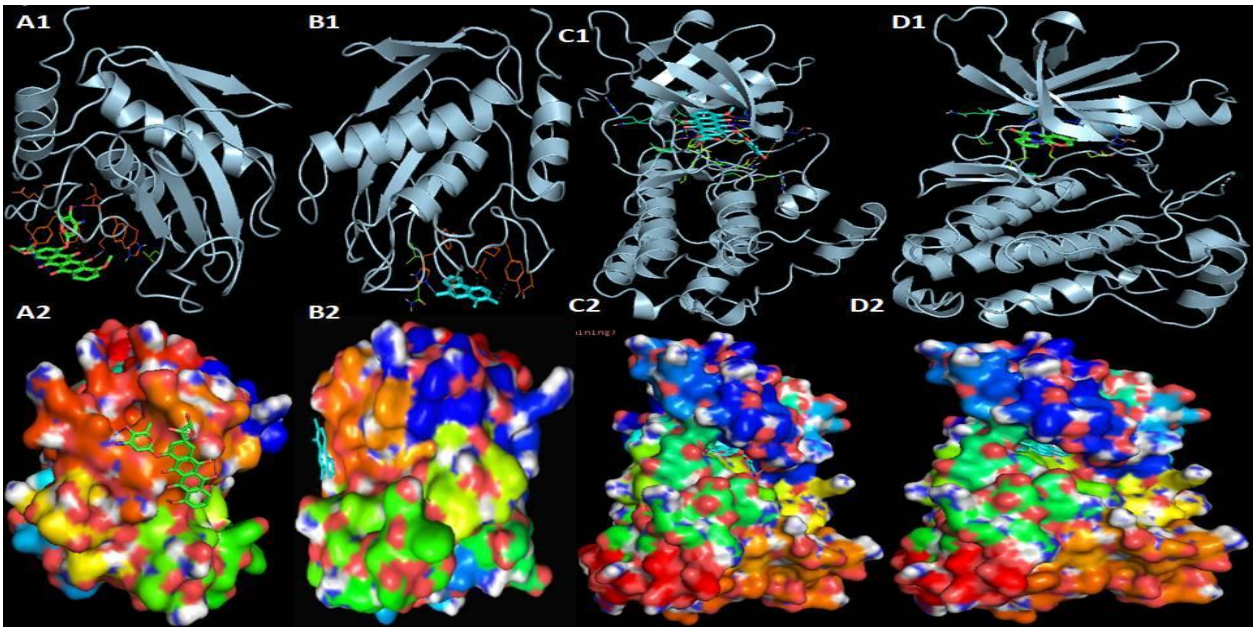


Fig: 2 Interaction and Best binding poses of ligands with 1BQO, 3PP0 protein of A549 and MCF-7 cell line

A1)1BQO - Doxorubicin structural interaction; A2) Best binding pose of 1BQO-Doxorubicin; B1) 1BQO-Fraction 3 structural interaction; B2) Best binding pose of 1BQO- Fraction 2; C1) 3PP0-Doxorubicin structural interaction; C2) Best binding pose of 3PP0-Doxorubicin; D1) 3PP0-fraction 3 structural interaction D2) Best binding pose of 3PP0-Fraction 3.

Conclusion:

The bioactive compound "7-(dimethyl amino) - 3ethyl-8 - oxo - 5 - thia - 1 - azabicyclo (4.2.0) -Oct - 2 -ene – 2 -carboxylic acid" (LS2) compound from *Bruguiera cylindrica* leaves showed a wide range of cytotoxic activity against A-549 and MCF-7 cell lines and the mixtures also showed potential outcomes i*n silico* in comparison with the standard drug doxorubicin. From this research, the researcher could deliberate the purified compound as a potential source for the development of strong anticancer agent.

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