Original Research Article

IN VITRO EVALUATION AND MOLECULAR DOCKING ANALYSIS OF POTENTIAL ANTICANCER COMPOUNDS FROM *SYZYGIUM ALTERNIFOLIUM*

Abstract

Around the world, *Syzygium alternifolium* is widely distributed in tropical and subtropical areas. This plant has traditionally been used to treat a variety of illnesses, including cancer. The current research compared the standard drug doxorubicin to the anticancer activity of methanolic extract of *Syzygium alternifolium* bark on human hepatocyte carcinoma (HepG2) cell line.Through DNA intercalation, inhibition of topoisomerase-II-mediated DNA repair, free radical production and consequent damage to cellular membranes, DNA, and proteins, doxorubicin exerts its anticancer activity in cancerous cells. Research on cytotoxicity have shown that *Syzygium alternifolium* phytoconstituents can selectively target cancer cells (IC_{50} = 185.585 µg/ml), while having little to no cytotoxic effects on normal cellsUsing Mcule docking software, molecular docking studies were performed against the human Topoisomerase-2 and CDK-2 proteins (Protein Data Bank-ID: 1ZXM and 1DI8, respectively). Seven compounds from the bioactives isolated are considered as safe inhibitors, according to *in silico* studies.In order to better understand the probable mechanisms of action and create more efficient and cost-effective therapies, molecular docking experiments were carried out employing phytoconstituents. This study demonstrates a significant consistency of anticancer therapeutic drug potentials of *Syzygium alternifolium* by *in vitro* and *in silico* approaches, leading the way for a better understanding of how integrating molecular docking and in vitro studies can improve the identification of cancer drugs.

Keywords:*Syzygium alternifolium*, Molecular docking, MTT assay, HepG2 cell lines.

1. Introduction

The two most prevalent types of primary liver malignancies (PLC) are hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), which account for 90% and 5% of cases, respectively [1, 2]. The prevalence of each type of cancer is the highest in the entire world. High yearly mortality rates are attributed to HCC, especially in endemic viral hepatitis areas like Thailand, Cambodia, and Laos [3]. Hepatocellular damage caused by reactive oxygen species (ROS) and the development of chronic inflammation linked to hepatocarcinogenesis occur prior to the induction of HCC [4].To treat HCC, adjunctive therapy have included tumour necrosis factor and melphalan, or cisplatin, epirubicin and 5-FU or doxorubicin, interferon alpha, and 5-FU [5]. The multi-drug resistance protein (MDR) is up-regulated and apoptotic proteins are down-regulated during chemotherapy treatment for HCC, which is the main issue [6]. In order to successfully treat cancer, apoptosis induction, which is the intended outcome, requires more effective chemotherapy [7].

An endemic aromatic tree, *Syzygium alternifolium* (Wt.) Walp. (*Myrtaceae*), is found in the Indian states of Andhra Pradesh and Assam. It is referred to as mogi/movi locally. For example, young shoots and fruits were used to treat dysentery, seeds were used to treat diabetes, and stem bark was used to treat gastric ulcers in traditional medicine [8]. Scientific research on the anti-cancer properties of *S. alternifolium* (Wt.) Walp (MESA) bark methanolic extract against MTT assay and *in silico* docking studies of isolated chemicals from the plant has not yet been published. The purpose of the current study is to investigate MESA's anti-cancer activity.

2. Materials and Methods

2.1 Plant materials

The bark of the *S. alternifolium* (Wt.) Walp plant was obtained in November 2014 in Tirupati, Andhra Pradesh, India's Sheshachalam highlands. The plant was subsequently authenticated and taxonomically recognised by botanist Dr. K. MadhavaChetty, an Assistant Professor at S.V. India's Tirupati University

2.2 Preparation of extract

The bark was mechanically ground into a coarse powder after being dried in the shade. The powder was sieved and kept in an airtight container while being extracted with 70% methanol at 75–78°C for up to 72 hours. Distillation was used to get rid of the solvent after extraction

was finished. The residue was a dark brown colour. The leftover material was concentrated before being placed in desiccators [9].

2.3 Method

MTT assay was performed for the test substances on HepG2 (Human Hepatocellular Carcinoma) cell line *in vitro*to determine the level of cytotoxicity.

2.4 Preparation of test compound for Cytotoxicity screening

To create a stock solution with a concentration of 10 mg/mL, 10 mg of test compounds were weighed and dissolved in DMEM-HG medium with 2% inactivated FBS. In order to provide lower concentrations for cytotoxicity testing, repeated two-fold dilutions of the stock solution were prepared [10].

2.5 Cell line and culture medium

The National Centre for Cell Sciences (NCCS), in Pune, provided the human hepatocellular carcinoma cell line. Stock cells were grown in DMEM-HG with 10% inactivated Fetal Bovine Serum (FBS), 100 IU/ml of penicillin, 100 µg/mL of streptomycin, and 5 µg/ml of amphotericin B at 37°C until confluent. TPVG solution (0.02% Trypsin, 0.02% EDTA, and 0.05% glucose in PBS) was used to dissociate the cells. All studies were conducted in 96 well microtitre plates, with the stock cultures being cultivated in 25 cm2 culture flasks (Tarsons India Pvt. Ltd., Kolkata, India).

2.6 Determination of Cell viability by MTT Assay

Using DMEM-HG with 10% FBS, the monolayer cell culture was trypsinized and the cell density was increased to 100,000 cells/ml. 0.1 mL of the dilute cell suspension was added to each well of the 96-well microtitre plate. After 24 hours, when a partial monolayer had developed, the supernatant was discarded, the monolayer had been washed with media once, and various test concentrations had been added. For comparison, the untreated cells were kept as a cell control.After 24 hours, the test solutions in the wells were discarded and 50 µL of MTT with DPBS was added to each well. The plates were then incubated at 37°C for another 24 hours in a 5% CO_2 atmosphere. Microscopic examination and observations were then conducted. The plates underwent a gentle shaking and a 3-hour incubation period at 37 °C with 5% CO_2 atmosphere. To dissolve the formazan that had developed, 100 µL of isopropanol was added after the supernatant was drained from the plates. At a wavelength of 570 nm, the absorbance was calculated using a microplate reader.

2.7Molecular Docking Studies

Initially the proteins were downloaded from PDB was prepared by removing extra chains. Attributes of spheres are prepared and noted. Later molecules drawn in molecular and ligand

preparation was created. Proteins are uploaded with sphere attributes and the structures were docked against 1DI8 and 1ZXM proteins. Docking indicated that some of our compounds have good binding ability with both CDK-2 and Human Topoisomerase-2 proteins. Following are the ligand interactions of compounds present in *Syzygium alternifolium*bark with 1DI8 and 1ZXM proteins [11].

2.7.1 Protein-ligand interactions

The binding orientation of drug candidates to their protein targets is predicted by docking stimulations. For creating docking simulation experiments, Mcule was employed.

2.7.2 Docking simulations on Cyclin-dependent kinase-2 (1DI8), Human topoisomerase II (1ZXM)

Cells advance into the S- and M-phases of the cell cycle under the control of the kinase cyclin-dependent kinase 2 (CDK2). Although CDK2 activity is mainly unnecessary for normal development, it is strongly linked to tumour growth in a variety of cancer types. Selective CDK2 inhibition continues to be attractive as a method to exploit in the development of anticancer drugs since it may have therapeutic benefits against some malignancies [12].Nuclear enzymes called DNA topoisomerases catalyse the addition of topological modifications to the DNA molecule. The DNA helix must be unwound in order for DNA to be replicated and translated, which creates helical tension throughout the rest of the DNA molecule. Topoisomerases are necessary for reproducing cells because they relieve this stress by creating temporary single-stranded (type I topoisomerases) or double-stranded (type II topoisomerases) fractures in the DNA double helix.

Hence, we report the molecular docking analysis of CDK2 and TOPO II with phytocompounds.

2.7.3 Ligand preparation

The 2D ligands sketched in Mcule docking in the ligand imported side.

2.7.4 Protein preparation

The x-ray crystallised structure of Cyclin-dependent kinase-2 (PDB ID: 1DI8) and Human topoisomerase II protein (PDB ID: 1ZXM) were retrieved from RCSB protein bank. Attributes of SBD site sphere are obtained from discovery studio visualizer.

2.7.5Ligand docking and scoring

Protein ligand interactions were stimulated though flexible glide-ligand docking with mCULE Docking allowed. The compounds docked displays a docking score

2.7.6 Visualization and analysis

Through the discovery studio visualizer, the resulting docking stances were seen. To understand the binding interactions between ligands and proteins, the ligand interactions were depicted. The glide score formula was used to select the best docked structures. The binding is more favourable the more unfavourable the score. Additionally, the various ligand receptor interactions were investigated as well as the docked ligand poses.

3. Results and Discussion

3.1 *In vitro* MTT assay

The findings showed that when bark extract concentrations increased, the rate of cytotoxicity increased as well. Based on the conversion of yellow tetrazolium MTT to a purple formazan dye by the mitochondrial dehydrogenase enzyme, the MTT assay measures the vitality of the cell. Therefore, the quantity of formazan generated was a direct reflection of the viability of metabolically active cells [13].The CTC50 values observed for Cell line HepG2 against methanolicextract of *Syzygium alternifolium* was 297.805 µg/ml given in table 1.Test substances were assayed for *in vitro* cytotoxicity study against HepG2 cells (Human Hepatocellular carcinoma) by exposing the cells to different concentrations ranging from 8 to 1000 µg/mL. MTT assay was employed to test the cytotoxic effect of selected concentrations of the plant extract on the cell viability of HepG2 cells by measuring the metabolic activity through a colorimetric determination. A population's percentage of healthy, alive cells is known as its cell viability. An increase or decrease in the number of viable cells is linearly proportional to mitochondrial activity since the MTT assay is typically used to identify cells with constant mitochondrial activity.In the present study, the CTC_{50} value of the plant extract was found to be 297.805 µg/mL. The plant extract exhibited 68.68±1.44% toxicity in HepG2 cells at the highest concentration tested (1000 µg/mL).

The plant's many parts are used to treat a wide range of ailments because of its extraordinary therapeutic qualities. Since quercetin directly promotes apoptosis in tumour cells, it can slow the spread of many types of human cancer. Numerous *in vitro* and *in vivo* investigations using a variety of cell lines and animal models have shown that quercetin has an anticancer impact.

However, quercetin has a significant toxicity against cancer cells while having little to no negative effects on healthy cells [14].Apigenin's anti-cancer properties and minimal toxicity have recently attracted a lot of attention. Apigenin has been shown to inhibit a number of human malignancies both *in vitro* and *in vivo* by a variety of biological mechanisms, including cell apoptosis and autophagy induction, cell cycle arrest, inhibition of cell migration and invasion, and immune response stimulation [15]. Kaempferol can cause anticancer effects by mechanistically inhibiting the expression of proteins implicated in the development and progression of cancer, as well as inducing apoptosis, arresting the cell cycle, and reducing the production of anti-inflammatory proteins [16]. Since the anticancer action of the extract may be caused by the presence of these chemicals in the extract.

Table 1: MTT Assay- Percentage cytotoxicity of MESA against Hep G2 cell lines

Test substance	**Concentration (µg/mL)**	**Percentage Cytotoxicity**	**CTC50 (µg/mL)**
Methanolic extract of *Syzygium alternifolium*	1000	68.68±1.44	297.805
	500	57.54±0.44	
	250	48.22±0.58	
	125	3.45±0.18	
	62.5	3.27±0.32	
	31.25	2.72±0.07	
	15.625	1.81±0.79	
	7.8	0.70±0.57	

Table 2: *In vitro* MTTassay –Percentage cytotoxicity of Doxorubicin against HepG2 cell lines

µM of Doxorubicin	**µg of Doxorubicin**	**Cytotoxicity %**	**CTC50 (µg/mL)**
10	5	86.28	
5	2.5	84.79	

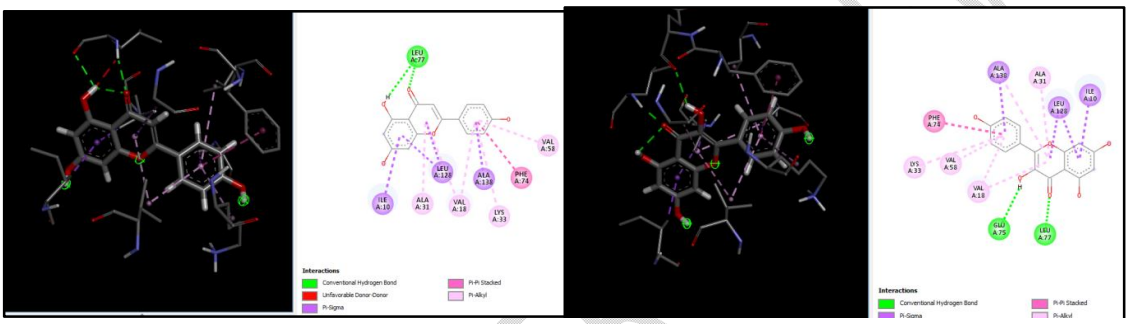
1	1.25	70.17	
0.5	0.625	57.47	0.485
0.25	0.312	41.63	
0.125	0.156	35.76	
0.0625	0.078	28.59	

Table 3: Molecular docking studies of isolated compounds against Topoisomerase-2 and CDK-2

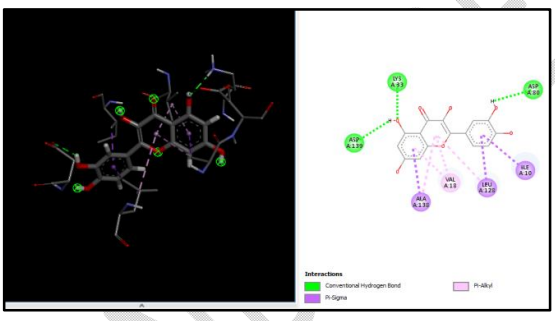
Compounds	**Cyclin Dependent Kinase II (1DI8) Docking Score**	**Topoisomerase II (1ZXM) Docking Score**
3-Hydroxy benzoic acid	-6.1	-7.2
Gentisic acid -	-5.7	-6.5
Caffeic acid	-7.1	--6.3
2,5-monoformal-1-rhamnitol	-5.5	-6.6
4-oxo-5-phenyl pentoic acid	-7.2	-7.2
Apigenine	-9.8	-9.4
Lutidine	-5.1	-5.1
Quercetin	-8.8	-9.8
Kaempferol	-9.4	-9.5
Acarbose	-6.2	-7.4
Squalene	-7.7	-8.3
Stigmasterol	-8.1	-8.6
Doxorubicin		

3.2 Molecular Docking Studies

Initially the proteins were downloaded from PDB was prepared by removing extra chains. Attributes of spheres are prepared and noted. Later molecules drawn in molecular and ligand preparation was created. Proteins are uploaded with sphere attributes and the structures were docked against 1DI8 and 1ZXM proteins. Docking indicated that some of our compounds have good binding ability with both Cyclin-dependent kinase-2 and Human topoisomerase II proteins represented in table 3. Following are the ligand interactions of compounds present in *Syzygium alternifolium*whole plant with 1DI8 and 1ZXM proteins represented in figure 1 and 2.



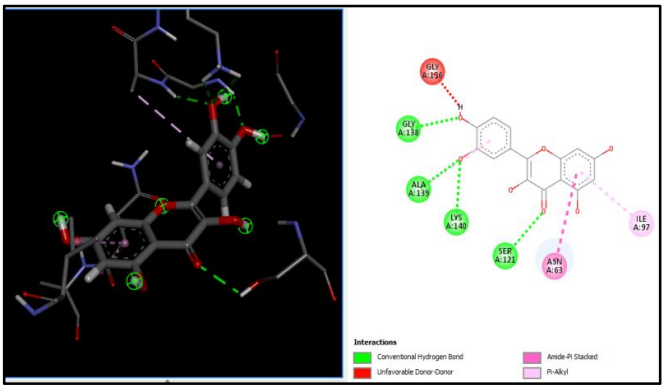
a) Apigenine -9.8 **b)Kaempferol -9.4**



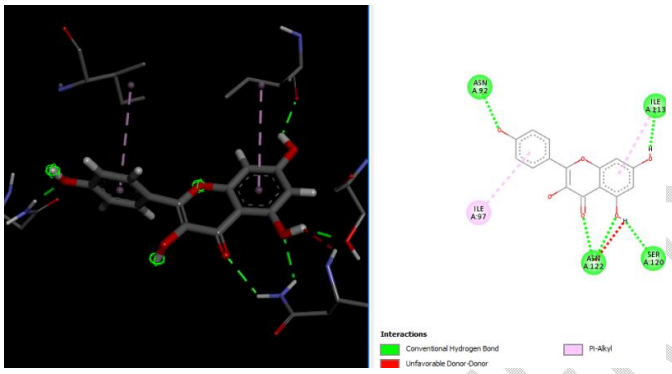
c) Quercitin -8.8

Figure 1: Docking poses of phytocompounds with Cyclin-dependent kinase-2

Quercetin - -9.8



Kaempferol - -9.5



Apigenine - -9.4

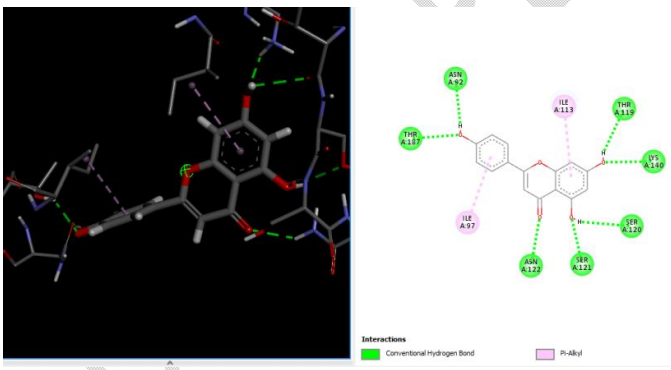


Figure 2: Docking poses of phytocompounds with Human topoisomerase II protein

This work demonstrates the importance of molecular docking in predicting the molecular interactions of phytocompounds with certain proteins. The pharmaceutical industry makes extensive use of this application as a potent tool, especially when analysing the link between structure and activity. In order to identify possible ligands, study of molecular docking

outputs—such as binding affinity—is routinely used. Small molecule ligands' capacity to bind to the proper target binding site can also be predicted via molecular docking.

Apigenine, Kaempferol and Quercitin have demonstrated remarkable binding affinity towards Cyclin-dependent kinase-2. Apigenine shown highest docking score -9.8 in comparison to other compound topwards CDK-2[17]**.** Quercetin, Kaemferol and Apigeninehave demonstrated remarkable binding affinity towards TOPO-2. Quercetin shown highest docking score -9.8 in comparison to another compound towards TOPO-2. Thus, indicating that these compounds are potent inhibitor of the CDK-2 antiapoptotic family of proteins and TOPO-2 proteins [18].

The application of molecular docking studies for the compounds that are present in methanolic extract of *Syzygium alternifolium* with proteins 1DI8 and 1ZXM are considered very useful and proven anticancer activity. According to our study, all phytocompounds may be able to inhibit the targeted 1DI8 and 1ZXM proteins due to their high binding affinities.

4. Conclusion

The *in vitro* MTT assay and *in silico* docking studies to predict anticancer activity were scientifically validated in the current study. The extract possesses anticancer activity, according to the MTT assay results, which were conducted. From docking scores, we can conclude that the isolated compounds from the extract possess anticancer activity through inhibition of CDK-2 and TOPO-2 can be further preceded to wet lab synthesis.

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