



## *In Silico* Molecular Docking Analysis of Flavone and Phytol from Vilvam (*Aegle marmelos*) against Human Hepatocellular Carcinoma (HepG-2) Mitochondrial Proteins



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**Abstract:** The vilvam fruit is an important source of phyto compounds, that are a good natural resource for curing several health illnesses. Annually, around 906,000 new cases and 830,000 deaths worldwide are attributed to liver cancer, making it one of the most prevalent malignant tumors. Humans' physical and mental well-being, as well as their social and economic advancement, are seriously threatened by and challenged by liver cancer. The molecular interactions between biological chemicals originating from plants and proteins relevant to apoptosis, however, are not well documented in research. Therefore, the objective of this study was to determine the potential biological compounds of flavone and phytol found in the vilvam fruit and examine their interactions with the targeted apoptotic proteins using molecular docking simulation towards liver cancer mitochondrial signalling pathway proteins such as Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C. Flavone showed a docking score with Caspase 3 (-10.51 kcal/mol), Bax (-9.49 kcal/mol), Bcl-2 (-11.10 kcal/mol), PARP (-10.22 kcal/mol) and p53 (-10.36 kcal/mol), but could not bind with Caspase 9 and Cytochrome C, while phytol could not bind with all the apoptotic proteins. The consequence of Lipinski rule recommends that flavone is the best curative drug for liver cancer. Docking results verify the application of flavone as a potential and natural therapeutic agent to treat diseases.

### Introduction

In practically every country, the annual death rate from cancer, a non-communicable disease, is rising. Globally, Globocan 2020 reported 19 million incidences of cancer and a death rate of around 10 million persons (Rajesh and Sivakumari, 2020). According to Maddika et al. (2007), mutations in tumor suppressor or oncogene genes usually affect the regulation of cell death and proliferation during tumor formation. The primary therapies for liver cancer consist of liver transplantation (Kardashian et al., 2020), chemotherapy (Hindson, 2020), radiotherapy (Zaheer et al., 2019), surgical resection (Tang et al., 2018), and local ablation therapy (Izzo et al.,

2019). Traditional medicines work well for liver cancer when it is still in its early stages, but they are less effective when the disease has progressed because of serious side effects, drug resistance, multiple recurrences, and metastases (Chuma et al., 2015; Xia et al., 2016; Wang et al., 2023). Along with surgery, radiation, hormone therapy, immunotherapy, and targeted therapy, chemotherapy is still one of the main treatments for treating the majority of malignancies (Hemalatha et al., 2020a; Padvathy et al., 2021; Mohammad Sitheek et al., 2023; Kulkarni et al., 2023; Saha and Yadav, 2023).

The success rate of chemotherapy in cancer treatment is not always satisfying; it has abundant side



effects and affects normal cells. That is why recent studies focused on the natural compound that has a cytotoxic effect on cancer cells without affecting normal cells (Bukowski et al., 2020; Nandana et al., 2023). Chemotherapy or radiation therapy resistance is caused by these acquired mutations and the ensuing changes in the related mitochondrial signalling pathways, the majority of the time, dose-limiting toxicities and substantial side effects are linked to current chemotherapy regimens (Evans, 2007; Petrylak, 2005). Therefore, finding drugs that target the programmed cell death pathway without harming healthy cells is essential for enhancing cancer treatment. Programmed cell death is categorized as type I apoptosis, type II autophagy, or type III programmed necrosis depending on morphological alterations. Programmed cell death is essential for controlling organism growth, tissue homeostasis, stress reactions, and the removal of damaged cells (Tan et al., 2009). The molecular changes, such as caspase and/or endonuclease activation, that lead to apoptosis include the externalization of phosphatidyl serine (PS), cell shrinkage, nuclear condensation, and finally DNA fragmentation Schwartzman and Cidlowski, 1993).

Drugs used in targeted therapy are directed at a particular biological target, such as a gene, a tissue environment, or a protein, and they stop a cell's unchecked proliferation. It is simple to examine the molecular interactions between targets and medications using computer-aided drug design techniques. It is one of the most recent methodologies that cover practically all phases of drug discovery. The fundamental objective of the method is to forecast how and how strongly the tiny molecule will bind to the target. Another benefit is that it takes less time, money, and risk to produce a medicine than conventional approaches do. A number of proteins involved in apoptosis, or programmed cell death, are crucial in the emergence and spread of cancer. It triggers through the sequential activation of two distinct pathways (Adams, 2003; Mala et al., 201).

One of the essential ingredients in Ayurvedic formulations is believed to be the powdered *Aegle marmelos* fruit. *A. marmelos* has also been shown to have protective properties against wounds, radiation, bacteria, the production of free radicals, and depression. These documents attest to *A. marmelos* inherent ability to heal. (Sarkar et al., 2020; Bhardwaj and Nandal, 2015). Current investigations and clinical trials conducted on the crude extracts of different plant parts have demonstrated their anti-inflammatory, anti-microbial, antiviral, anticancer, chemopreventive, antipyretic, analgesic, anti-ulcerative, diuretic, and anti-diarrheal qualities

(Choudhary et al., 2021). Several beneficial phytoconstituents have been found in *A. marmelos* fruit, including limonene,  $\alpha$ -phellandrene, betulinic acid, marmesin, luvangentin, auroptene, rutaretin, scopoletin, aegeline, umbelliferone, marmelin, fagarine, and anhydromarmelin. According to a study, the plant's fruit pulp possesses a significant amount of riboflavin and tannins (Reddy and Urooj, 2013; Bheeman et al., 2014; Rahman and Parvin, 2014; Tiwari et al., 2022). To determine the potential of particular GC-MS spectrum components from *A. marmelos* methanol fruit pulp extract, such as flavone and phytol, against apoptotic proteins, *in silico* molecular docking experiments were conducted in light of this.

## Materials and Methods

### In Silico Docking Studies

The mode of action of the ligands, flavone and phytol when docked against Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C proteins was predicted by *in silico* molecular docking studies.

### Retrieval of Protein Sequence and Structure

The proteins sequence of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C were retrieved from Swiss-Prot database. The protein structures of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C were downloaded from PDBSum database. The 3-D structure of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C were visualized using RasMol Tool.

### Docking Analysis of Ligand with Proteins

The 2-D structure of flavone and phytol was drawn and converted to 3-D structure using ChemSketch and then, converted to 'mol' format. ArgusLab is a molecular modeling, graphics, and drug design program for Windows operating systems were used. To date, there are >20,000 downloads. We will be able to include in our model several atoms, residues, groups and calculations. PyMol Viewer software was used to visualise the docked structures of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53, and Cytochrome C with flavone and phytol in order to forecast the outcomes.

## Results and Discussion

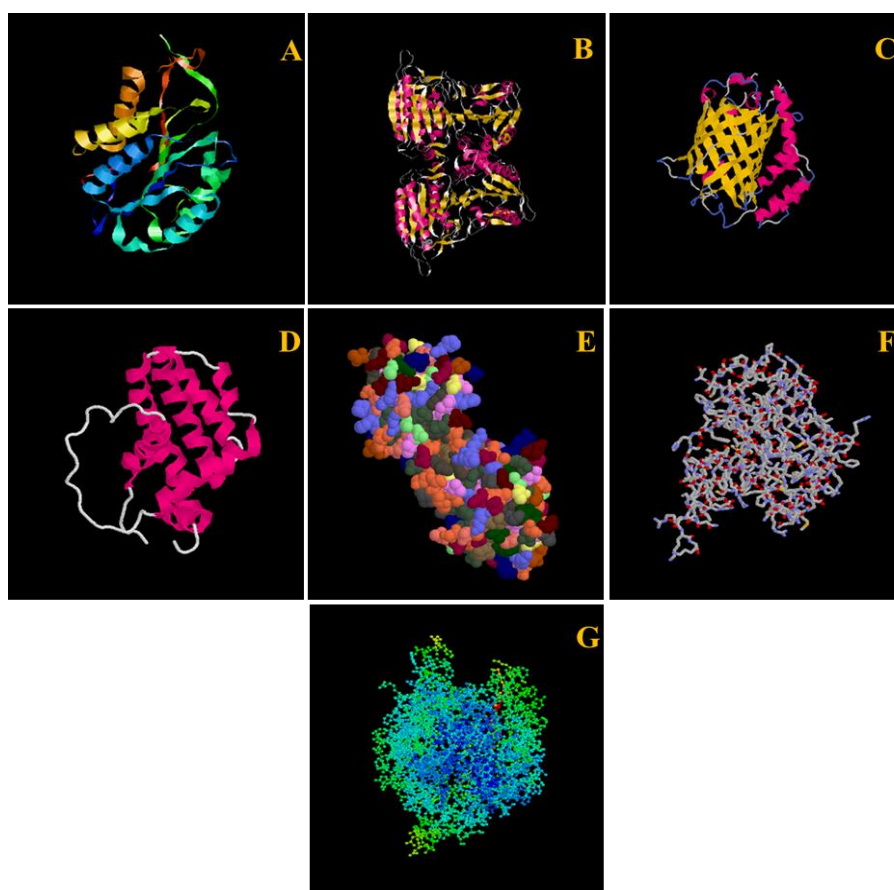
The primary goal of anti-cancer treatments is to eliminate the cancer cells without affecting the normal cells, which can be very difficult to obtain by chemotherapy or radiotherapy, Chemotherapy drugs such as doxorubicin have several adverse effects like bone marrow suppression, nausea or vomiting, hair loss, and cardiotoxicity (Lipinski, 2004; Tacar et al., 2013; Hassan

et al., 2017). Natural components play an important role in chemotherapy, where a variety of phytochemical ingredients may regulate apoptosis-linked signalling pathways and improve the effectiveness of cancer chemotherapy (Browning et al., 2010). Chemotherapy resistance arises in cancer-infected cells as a result of resisting some potential apoptosis mechanisms, such as reduction of pro-apoptotic signals, strengthening of anti-apoptotic signals, and poor apoptosis instigation and application. Although lately claimed to be free from the intricacy in the molecular process, the functional connection between apoptosis remains complex despite this. Therefore, modifying important components of the apoptotic pathways may be a novel therapeutic strategy for enhancing the overall efficacy of cancer treatment (Chirumbolo, 2012).

The therapeutic efficacy of cancer patients can be improved while adverse effects can be decreased thanks to phytochemicals and their derivatives. These phyto-constituents include a variety of physiologically active substances that are present in nature and have potent anticancer properties. Phytochemicals frequently function by regulating the molecular signalling pathways that are thought to be involved in the growth of cancer. The specific processes include enhancing antioxidant status,

inactivating carcinogens, suppressing proliferation, starting cell cycle arrest and apoptosis, and regulating the immune system (Choudhari et al., 2020).

Apoptosis, also known as programmed cell death, is a tightly regulated system that eliminates undesired, aged, and damaged cells (Yang et al., 2006). The most significant and well-known signs of apoptosis are morphologic modifications, such as a decrease in cell membrane thickness, a shrinkage of the heterochromatin core, and a loss of organelle positioning in the cytoplasm. Advanced molecular processes and mechanisms are also involved in addition to significant morphological alterations. Apoptosis-inducing system is produced by the alignment of several processes and molecular alterations. Extrinsic pathway and intrinsic pathway are the two main processes that contribute to the induction of apoptosis. The intrinsic mechanism is mitochondrial-mediated, whereas the extrinsic pathway is death receptor-mediated (HemaIswarya and Doble, 2006; Elmore, 2007; Goldar et al., 2015). To design neoplastic treatments, it is crucial to recognize the factors that contribute to cancer development. A key indicator of cytotoxic anticancer drugs is the induction of apoptosis. It has been demonstrated that certain natural substances, such as those found in plants, cause apoptotic pathways to be

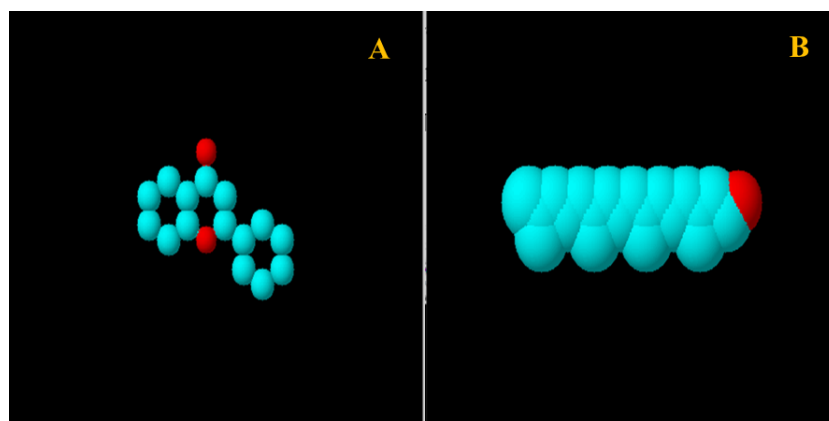


**Figure 1. 3 D structure of proteins visualized by RasMol Tool.**

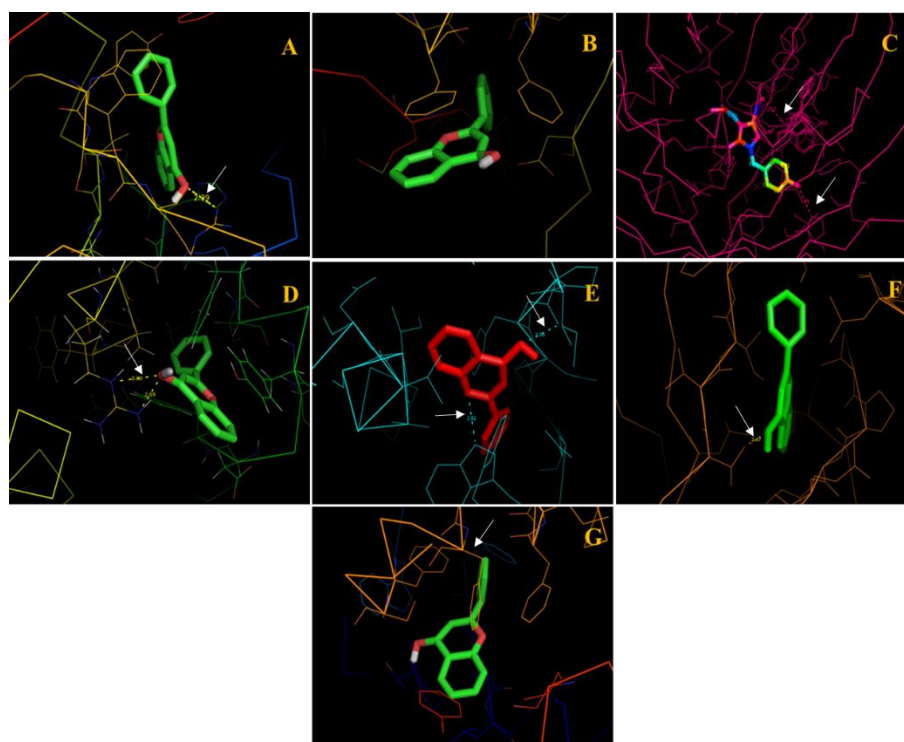
**(A) Caspase 3 (B) Caspase 9 (C) Bax (D) Bcl-2 (E) PARP (F) p53 (G) Cytochrome C**

stopped in cancer cells (de Araújo Júnior et al., 2012). Studies have shown that natural herbal remedies have the ability to treat a wide range of human ailments, including cancer. According to several studies, herbal cocktails,

In the present molecular docking analysis of ligands against apoptotic proteins, flavone and phytol were docked against Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome C. The protein sequences of Caspase



**Figure 2. Structure of Ligands (A) Flavone (B) Phytol**

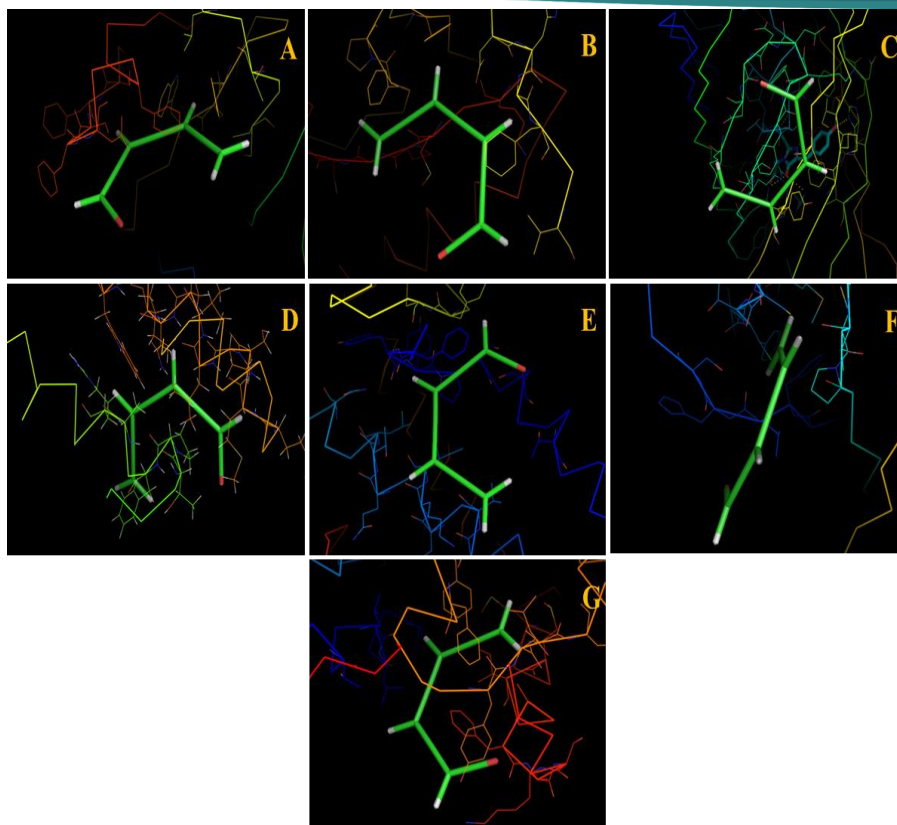


**Figure 3. Docked structure of flavone with apoptotic proteins.**  
**(A) Caspase 3 (B) Caspase 9 (C) Bax (D) Bcl-2 (E) PARP (F) p53 (G) Cytochrome C.**

which are multi-herb mixes delivered in a single recipe, may work to enhance the therapeutic efficacies of each herbal component (Kiyohara et al., 2004; Corson and Crews, 2007; Kim et al., 2014). In addition to the apoptotic research, docking studies were done to determine the docking capability of two particular GC-MS spectrum phytocompounds found in *A. marmelos*, flavone and phytol, against Caspase-3, Caspase-9, Bax, Bcl-2, PARP, p53, and Cytochrome C proteins.

3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome C were retrieved from Swiss-Prot; their accession numbers are: 1CP3, 1JXQ, 4BDU, 1G5M, 5DSY, 1GZH and 5Z62. The structure of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome C was downloaded from PDBSum and the PDB IDs are: 1CP3, 1JXQ, 4BDU, 1G5M, 5DSY, 1GZH and 5Z62. The 3D structure of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome C was visualized using RasMol (Figure 1).





**Figure 4. Docked structure of phytol with apoptotic proteins**  
 (A) Caspase 3 (B) Caspase 9 (C) Bax (D) Bcl-2 (E) PARP (F) p53 (G) Cytochrome C.

**Table 1. Interactions between ligand flavone and phytol with Caspase 3, Caspase 9, Bax, Bcl-2, p53, PARP and Cytochrome C proteins.**

Proteins	Ligands	Docking Score (Kcal/Mol)	Distance (Å)
Caspase 3	Flavone	-10.51	1
Caspase 9		No Interaction	
Bax		-9.49	6
Bcl-2		-11.10	2
PARP		-10.22	2
p53		-10.36	1
Cytochrome C		No Interaction	
Caspase 3	Phytol	No Interaction	
Caspase 9		No Interaction	
Bax		No Interaction	
Bcl-2		No Interaction	
PARP		No Interaction	
p53		No Interaction	
Cytochrome C		No Interaction	

The structure elucidation of flavone and phytol was done using ChemSketch. The 3-D structure of flavone and phytol was drawn using ACD/ChemSketch in 'mol' format and converted to 'PDB' format using Open Babel (Figure 2). The ligands, flavone and phytol were docked with the 3D structure of Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome C by ArgusLab. In silico molecular docking, the study revealed the interactions between ligands and proteins in order to calculate the

binding energy between them. The results showed that there is a presence of a binding site between these proteins and the ligand. The docking is also valid by the formation of hydrogen bonds between them. The docked complex of flavone and phytol with Caspase 3, Caspase 9, Bax, Bcl-2, PARP, p53 and Cytochrome-C were visualized by PyMol (Figure 3 and Figure 4).

This result shows that there is a presence of a binding site between the protein "Caspase 3, Bax, Bcl-2, PARP

and p53" and ligand flavone, whereas there is no binding site between the proteins and ligand phytol. The docking is also valid by the formation of hydrogen bonds between them. The result of Lipinski rule suggests the analysed compound (flavone) as the best therapeutic drug. Docking study and *in silico* docking results proves the application of flavone as potential and natural therapeutic agents to treat disease (Table 1).

When docking was carried out among the seven proteins and the ligands flavone and phytol, the docked outcome showed that flavone established to cover high-quality binding affinity with five apoptotic proteins (Caspase 3, Bax, Bcl-2, PARP and p53), but could not bind with two apoptotic proteins (Caspase 9 and Cytochrome C), while phytol could not bind with all the seven apoptotic proteins. *In toto*, flavone docks well to these five proteins responsible for disease and is said to be the best compound. The rule of Lipinski suggests the analysed flavone is the best therapeutic drug. Docking study results prove the application of flavones GC-MS spectral compound from *A. marmelos* as a potential and natural therapeutic agent to treat diseases.

In our docking results, the ligands flavone and phytol, the docked outcome showed that flavone was established to cover high-quality binding affinity with five apoptotic proteins (Caspase 3, Bax, Bcl-2, PARP and p53). Flavone with Caspase 3 showed a docking score of -10.51 kcal/mol and had 1 hydrogen bond, with Bax showed a docking score of -9.49 kcal/mol and having 6 hydrogen bonds, with Bcl-2 showed a docking score of -11.10 kcal/mol and having 2 hydrogen bond, with PARP showed a docking score of -10.22 kcal/mol and having 2 hydrogen bond and with p53 showed a docking score of -10.36 kcal/mol and having 1 hydrogen bond, but could not bind with two apoptotic proteins (Caspase 9 and Cytochrome C), while phytol could not bind with all the seven apoptotic proteins.

Similar docking studies of stearic acid present in *Cardiospermum halicacabum* with HepG-2 cell line protein plasminogen and transferrin (Rajesh et al., 2016), GC-MS spectral compounds in propolis against apoptotic proteins (Caspase 3, Caspase 9 and  $\beta$ -actin) (Flora Priyadarshini et al., 2018), rutin compound against apoptotic proteins (TNF, Caspase 3, NFKappa-B, p53, Collagenase, Nitric Oxide Synthase and Cytochrome C) (Jayameena et al., 2018), alginic acid and fucoidan compounds present in *Sargassum wightii* against apoptotic proteins (Caspase 3, Caspase 9 and  $\beta$ -actin) (Jayaprakash et al., 2018), ascorbic acid, betalain and gallic acid that are present in dragon fruit against apoptotic proteins Caspase 9 and  $\beta$ -actin) (Karthika et al., 2018), pure

propolis compound with Caspase 3, Caspase 9, Bax, Bcl-2 and BclxL (Rajini Selvaraj et al., 2019), molecular docking studies of apoptotic proteins Caspase 3, Caspase 9, bax, bcl-2 and bcl-xl with ethyl (2s)-2-methylbutanoate and 1-(ethylsulfanyl) ethane-1-thiol from durian fruit (Mohamad Sitheek et al., 2020) and isolated compound from kiwi fruit with apoptotic proteins (Caspase 3 and  $\beta$ -Actin) (Ashok et al., 2021). Likewise, the docking interaction of three compounds (Muricin J, K, and L) from *Annona muricata* with apoptotic proteins (Caspase 3, Caspase 9 and  $\beta$ - Actin) (Hemalatha et al., 2020b) and Squalene and Rhodoxanthin (Selected GC-MS spectral compound from *Hylocerus undatus* methanol fruit pulp extract) docked against AIF, APAF-1, BAK, Caspase 8 and RIP proteins (Padmavathy et al., 2022). These findings support the results of the present work.

The negative side effects of synthetic pharmaceuticals necessitate the development of new and better medications. A biological activity demonstrates that the methanol fruit pulp extract from *A. marmelos* contains potential anticancer compounds. As a result, we conducted a study to determine the binding affinity of specific GC-MS spectrum compounds from the methanol fruit pulp extract of *A. marmelos*, which may be a potential lead molecule. The use of computational biology and bioinformatics has the potential to alter how pharmaceuticals are designed as well as speed up the drug development process and cut expenses. A variety of techniques are used to identify novel compounds throughout the drug-developing process, which is facilitated and sped up by rational drug design (RDD). The drug molecule docking with the receptor (target) is one such technique. The receptor is the site of pharmacological action, which is ultimately in charge of the therapeutic impact (Richon, 1994).

However, apoptosis is a complicated process that involves a large number of proteins, mediators, signals, genes, pro- and anti-apoptotic proteins, and pathways. We support more research on flavone in diverse apoptotic signals, mediators, indicators, routes, and proteins in *silico*, *in vitro*, and particularly *in vivo*. Numerous natural chemicals remain to be investigated for their potential as cancer treatments and numerous apoptotic pathways require additional research.

## Conclusion

In this study, molecular docking was carried out to determine the binding affinity of flavones and phytol with HepG-2 mitochondrial proteins and to associate with its docking score. The outcomes are helpful for scheming and evolving a novel drug that has enhanced inhibitory

activity against liver cancer. From this study, we conclude that flavone is the best inhibitor for Caspase 3, Bax, Bcl-2, PARP, and p53. This study should increase scholars' interest in new areas of investigation. This potential drug candidate awaits further justification by wet lab investigations for its accurate function as an anticancer drug.

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### Conflict of Interest

The authors declare no conflict of interest.

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