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Research Article

Exploring Squalene and Rhodoxanthin from *Hylocereus undatus* as a Therapeutic Agent for the Treatment of Human Liver Cancer using Docking Analysis

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A B S T R A C T

Introduction: The dragon fruit *H. undatus* contains several active phyto-compounds, which act as good antioxidant, anti-inflammatory, and anti-cancer agents. To explore the apoptotic potential of these phyto-compounds, it would be apt to screen the interaction of these compounds with apoptotic proteins via., *in silico* docking studies.

Method: The current study was planned to evaluate the docking interaction of selected GC-MS spectrum components from *H. undatus* (squalene and rhodoxanthin) with apoptotic proteins (AIF, Apaf-1, BAK, caspase 8, and RIP) by PatchDock docking algorithm.

Results: The docking interaction showed that rhodoxanthin docks well with apoptotic proteins, and rhodoxanthin is recommended by the Lipinski rule as the best treatment for liver cancer.

Conclusion: The application of rhodoxanthin as a potential and natural therapeutic agent to cure diseases is validated by docking results.

Keywords: Dragon Fruit, Squalene, Rhodoxanthin, Apoptotic Proteins, Liver Cancer

Introduction

One of the most feared diseases of the 20th century, cancer, is still growing and becoming more common in the 21st century. Uncontrolled cell division that results in aberrant cell proliferation characterises the disorder. About 6.7 million individuals worldwide pass away from cancer every year. Around 270,000 new cases and 145,000 deaths are reported annually globally, with two-thirds of them occurring in third-world nations. It is viewed as a rival to modernity and an advanced form of socio-cultural life that is controlled by western medicine.[1] Although significant attempts are being made to combat this disease by multidisciplinary clinical investigations, the best and most definitive treatment needs to be included in global pharmaceutical products. The study of complementary and alternative medicines that aid in the treatment of most malignancies has received increased attention recently. Under a great number of ethnobotanical grounds, several herb studies were conducted.

A well-liked method for finding new leads is computational

drug design. The use of virtual screening techniques cuts down on both the cost and time associated with drug discovery. With the aid of those computational modelling tools, more than 50 tablets were created and recycled, and several of them, including raltegravir, saquinavir, nelfinavir, and itraconazole were given FDA approval for marketing and advertising.[2] The goal of molecular docking is to predict how a ligand molecule will behave, interact, and conform when it binds to its target molecule, which is often a huge macromolecule. Every bound ligand is assigned a scoring function by the software with a predetermined procedure after calculating the type of interactions; this function reflects the binding affinity. The best interaction between a ligand and a receptor is represented by the lowest binding score.[2-5]

Natural goods have traditionally and constantly been investigated for promising new leads in pharmaceutical development. Researchers are continually discovering new antitumour drugs and anticancer pills based on studies involving plants. In view of this, structure-based *in silico* molecular docking has been carried out to discover the docking potential of selected GC-MS spectral compounds *viz.*, squalene and rhodoxanthin present in *H. undatus* against apoptotic proteins.

Materials and Method

In Silico Docking Studies

Our previous GC-MS spectral analysis reported that squalene (RT-48.194) and rhodoxanthin (RT-54.175) are present in *H. undatus* fruit pulp methanol extract.[6] Selected GC-MS spectrum components from the methanol fruit pulp extract of *H. undatus*, such as squalene and rhodoxanthin, were docked against the proteins AIF, Apaf-1, BAK, caspase 8 and RIP to anticipate the mode of action of the ligands based on our prior findings.

Protein Sequence Retrieval - Swiss-Prot

The sequence of AIF, Apaf-1, BAK, caspase 8 and RIP proteins were retrieved from the Swiss-Prot database.

Protein Structure Retrieval - Protein Data Bank

The structures of AIF, Apaf-1, BAK, caspase 8 and RIP were downloaded from the PDBSum database.

Structure Visualization - RasMol

The 3-D structures of AIF, Apaf-1, BAK, caspase 8 and RIP were visualised using RasMol Tool.

Three-dimensional Structure of Inhibitor - ChemSketch

The 2-D structure of squalene and rhodoxanthin were drawn and converted to a 3-D structure using ChemSketch and then, converted to 'mol' format.

Conversion of Ligand Format - Open Babel

Open Babel which is a chemical toolbox, contains all chemical data, which can be searched, analysed, converted, and stored. Since the downloaded AIF, Apaf-1, BAK, caspase 8 and RIP protein structures are in PDB format, the ligands squalene and rhodoxanthin which are in 'mol' format have also to be converted into PDB format. For this purpose, the Open Babel tool was used.

Docking - PatchDock

The apoptotic proteins AIF, Apaf-1, BAK, caspase 8 and RIP were docked with the ligands squalene and rhodoxanthin using the PatchDock docking tool.

Visualisation of Protein - PyMol Viewer

The docked structures of AIF, Apaf-1, BAK, caspase 8 and RIP with squalene and rhodoxanthin were visualised using PyMol viewer software to predict the results.

Results

In Silico Docking Studies

For molecular docking analysis of ligands, squalene and rhodoxanthin were docked against the apoptotic proteins *viz.*, AIF, Apaf-1, BAK, caspase 8 and RIP.

Sequence Retrieval - Swiss-Prot

The proteins of AIF, Apaf-1, BAK, caspase 8 and RIP were retrieved from Swiss-Prot; their accession numbers were: P55008, O14727, Q16611, Q14790 and Q13546.

Structure Retrieval - Protein Data Bank

The structures of AIF, Apaf-1, BAK, caspase 8 and RIP were downloaded from PDBSum and the PDB IDs were 2D58, 1CY5, 2IMS, 1I4E and 4ITH.

Structure Visualization - RasMol

The 3-D structures of AIF, Apaf-1, BAK, caspase 8 and RIP were visualised using RasMol (Figure 1).

Three-dimensional Structure Elucidation of Inhibitor – ChemSketch

The structure elucidation of squalene and rhodoxanthin was done using ChemSketch. First, the 2-D and 3-D structures of squalene and rhodoxanthin were drawn using ACD ChemSketch in 'mol' format and were then converted to 'PDB' format using Open Babel (Figures 2 and 3).

Molecular Docking - PatchDock

The ligands, squalene and rhodoxanthin were docked with 3-D structures of AIF, Apaf-1, BAK, caspase 8 and RIP by the PatchDock algorithm. The *in silico* molecular docking study showed the interactions between ligand and proteins in order to calculate the Geometrical Shape Complementarity Score (GSCS) between them.

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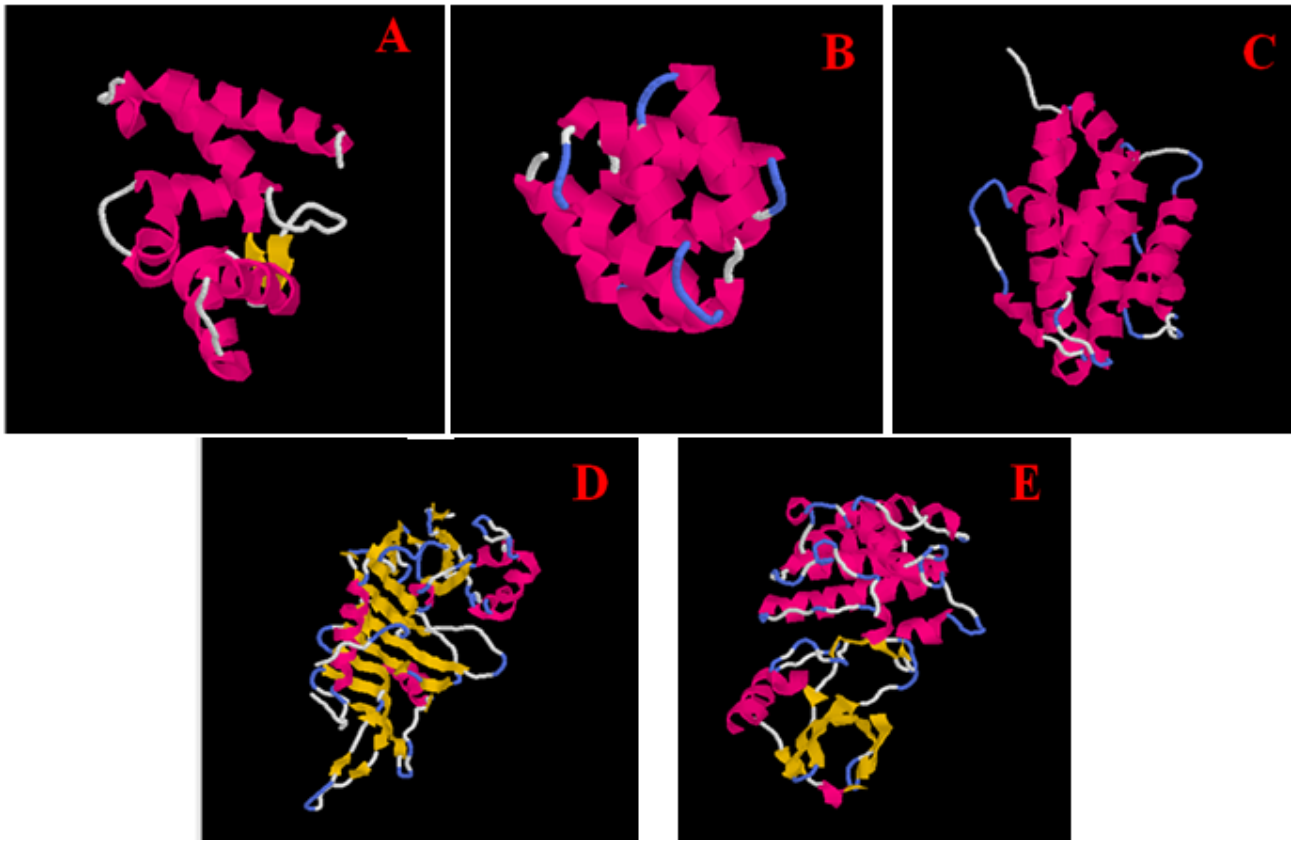

Figure 1.3-D Structures of Proteins visualised by RasMol Tool
(A) AIF, (B) Apaf-1, (C) BAK, (D) Caspase 8, (E) RIP

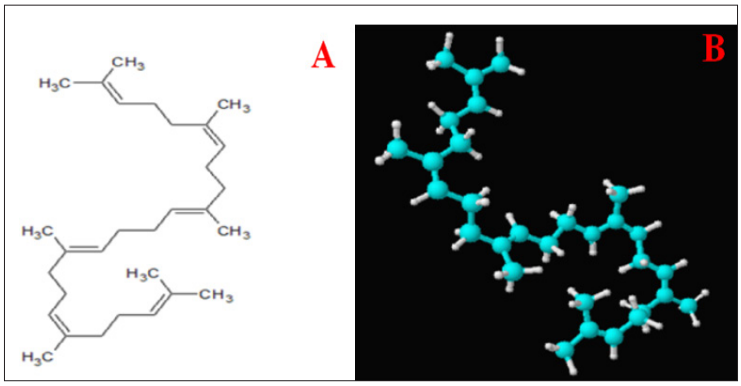

Figure 2.Structures of Squalene (A) 2-D Structure, (B) 3-D Structure

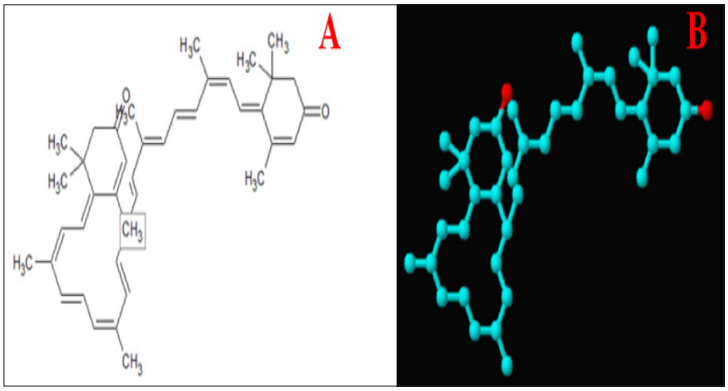

Figure 3.Structures of Rhodoxanthin (A) 2-D Structure, (B) 3-D Structure

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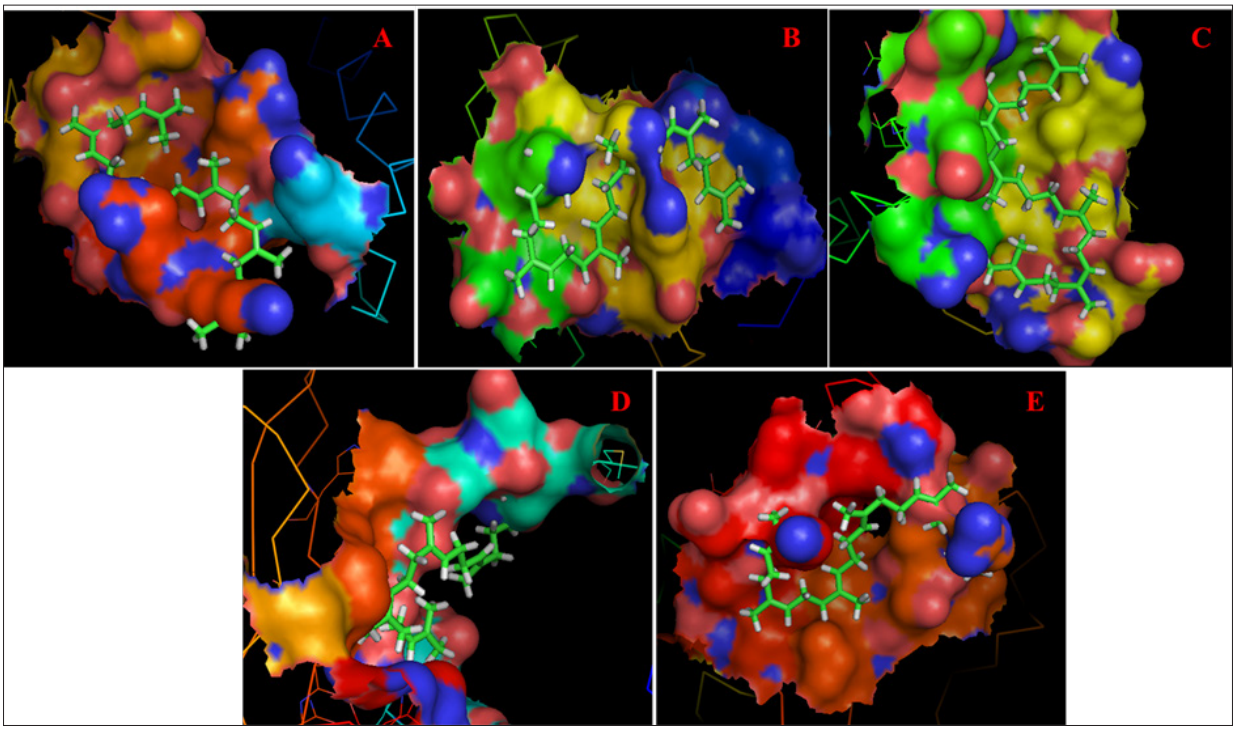

Figure 4.Docked Structure of Squalene with Apoptotic Proteins
(A) AIF, (B) Apaf-1, (C) BAK, (D) Caspase 8, (E) RIP

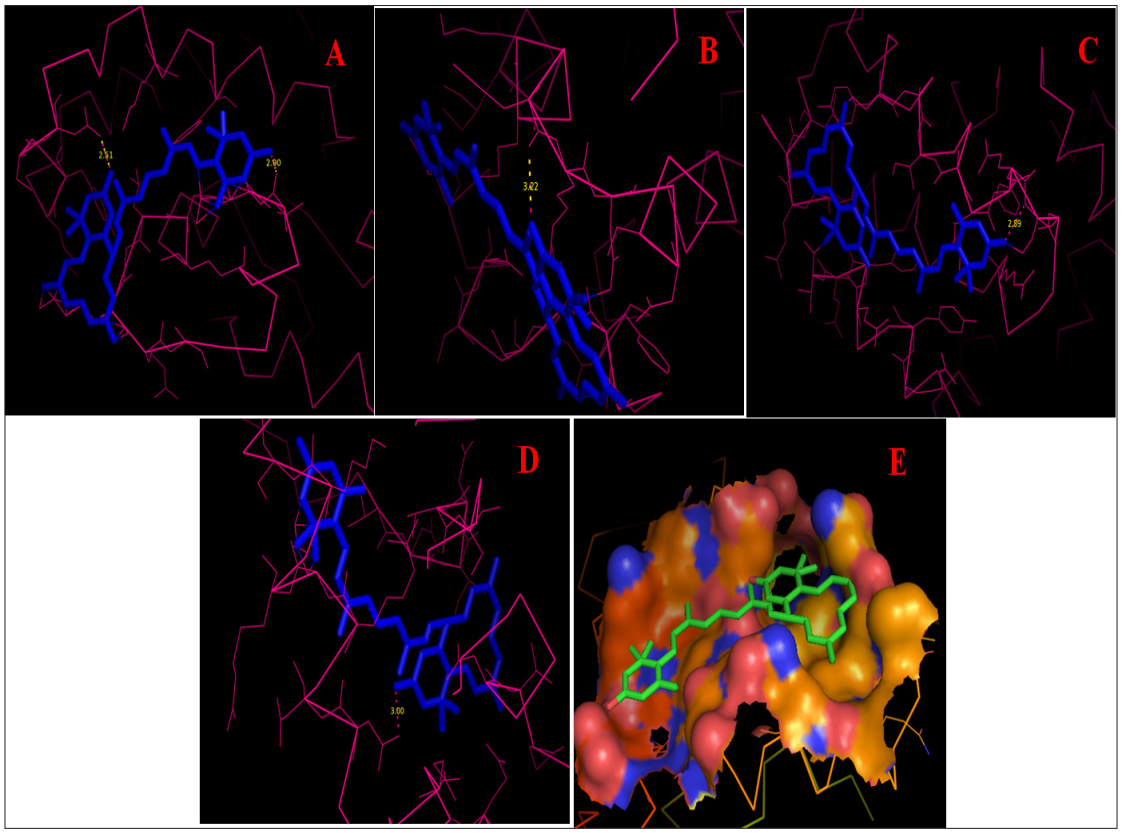

Figure 5.Docked Structure of Rhodoxanthin with Apoptotic Proteins
(A) AIF, (B) Apaf-1, (C) BAK, (D) Caspase 8, (E) RIP

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Table 1.Interactions between Ligand Squalene and Rhodoxanthin with AIF, Apaf-1, BAK, Caspase-8 and RIP Proteins

Ligand	Protein	Geometrical Shape Complementarity Score (GSCS)	H-Bond
Squalene	AIF	No interaction	
	Apaf-1	No interaction	
	BAK	No interaction	
	Caspase 8	No interaction	
	RIP	No interaction	
Rhodoxanthin	AIF	6248	2
	Apaf-1	5532	1
	BAK	6088	1
	Caspase 8	6710	1
	RIP	No interaction	

The results showed the presence of a binding site between these proteins and the ligand. The formation of the hydrogen bond between them serves as additional evidence supporting the docking (Table 1).

Visualisation of Docked Complex by PyMoltool

The docked complex of squalene and rhodoxanthin with AIF, Apaf-1, BAK, caspase 8 and RIP were visualised by PyMol (Figures 4 and 5).

From the below docking results, the ligand squalene when docked with AIF, Apaf-1, BAK, caspase 8 and RIP showed no interaction, indicating that, there is no specific protein-ligand binding site in the sequence; rhodoxanthin with RIP also showed no interaction.

On the other hand, rhodoxanthin with AIF showed a GSCS for 6248 and had 2 hydrogen bonds, with Apaf-1, it showed a GSCS for 5532 and had 1 hydrogen bond, with BAK, it showed a GSCS for 6088 and had 1 hydrogen bond, and with caspase 8, it showed a GSCS for 6710 and had 1 hydrogen bond (Table 1).

When docking was carried out among the five proteins and the ligands squalene and rhodoxanthin, the outcome revealed that rhodoxanthin showed a high-quality binding affinity with four apoptotic proteins (AIF, Apaf-1, BAK and caspase 8), but could not bind with one apoptotic protein (RIP), while squalene could not bind with all the five apoptotic proteins.

In toto, rhodoxanthin is thought to be the best molecule since it binds strongly to the four proteins that cause the disease. The examined rhodoxanthin is recommended as the best therapeutic medicine according to Lipinski's guidelines. The result of the current docking investigation proves the application of rhodoxanthin GC-MS spectral compound from *H. undatus* as a potential and natural therapeutic agent to treat diseases.

Discussion

Cancer is characterised by abnormal cell proliferation that interferes with normal bodily processes. The majority of patients who suffer from this terrible disease die soon because of inadequate, sophisticated treatments.[2] The authors also reported that chemotherapeutic treatments, for example, have additional post-treatment problems that render them unsuitable for prolonged usage. Numerous phytochemicals found in medicinal plants have considerable therapeutic significance and can kill cancer cells. These substances function in a variety of ways, but they often display their anticancer potential by blocking several proteins essential for cell growth and division. By offering a simulated biological system, the computational method known as molecular docking makes it easier to identify the optimal molecule from a group that may attach with the maximum affinity with the desired target. This procedure employs a scoring mechanism to score the molecules that fit the target and operates on the basis of a specific algorithm.[2]

Earlier, anticancer drugs were discovered via chemical agents, however, effective chemopreventive agents were discovered in natural materials. In clinical and preclinical trials, testing a potential drug's safety and efficacy is of paramount importance, whereas *in vitro* and *in vivo* assessment of toxicity and safety is expensive and time-consuming, *in silico* ways to analyse the drug properties have made the test quite simpler.[2] Molecular docking determines a ligand's optimum potential position within the confines of a receptor molecule's binding site and computes binding energy.[7] The formation of hydrogen bonds between the ligand and receptor makes the connection more specific, which enhances molecular recognition and interaction strength.[8] According to the degree of binding in this instance, the rhodoxanthin ligand molecule generated

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a sizable number of hydrogen bonds inside the binding site of the receptor molecules.

One of the most significant GC-MS spectrum compounds, rhodoxanthin has been discovered in dragon fruits, particularly those with a retro structure of carotenoids.[6] Rarely occurring in nature, rhodoxanthin has been observed in various fish and bird feathers in the past.[9] Carotenoids have lately developed from simple chemicals that interact with radical species to biomarkers connected to the treatment of a number of degenerative diseases, such as age-related macular degeneration, lung, gastrointestinal system, and pancreatic, breast, and prostate cancers.[10,11]

It has been demonstrated that certain natural carotenoids have anti-carcinogenic properties. According to epidemiological studies, eating green and yellow fruits and vegetables reduces your risk of developing cancer. These fruits and vegetables are rich in beta-carotene, which has been intensively researched as a potential cancer preventive agent, but certain carotenoids that co-exist with beta-carotene in fruits and vegetables also have anti-carcinogenic properties.[12] As a result, several researchers[12] have conducted more in-depth research on the cancer-preventing properties of the natural carotenoids found in foods, specifically lutein, lycopene, zeaxanthin, and beta-cryptoxanthin. However, there are no reports of rhodoxanthin being effective against cancer. Analysis of the action mechanism of this natural carotenoid is currently underway, and some intriguing results have already been achieved.

Small, sealed membrane vesicles known as apobodies or apoptotic bodies are created when cells experience apoptosis, a kind of cell death. Apoptosis is a mechanism that stops tissue damage, inflammatory or autoimmune reactions, and the release of potentially harmful or immunogenic cellular contents from dying cells.[13,14] Therefore, macrophages, cancerous cells, and parenchymal cells phagocytize apoptotic bodies, which are then corrupted within phagolysosomes. No inflammatory reactions associated with apoptosis take place since the apoptotic cells do not release their cellular components into the nearby interstitial tissue.[15,16]

The cysteine protease family known as caspases serves as an important catalyst for the vital hydrolytic processes of apoptosis, necrosis, and inflammation.[17-19] In most cells, caspases are widely produced in an inactive proenzyme form that can frequently activate other pro-caspases and start a protease cascade. Two significant apoptotic mechanisms have been identified: the mitochondrial (intrinsic) and death receptor (extrinsic) pathways.[20-22] These mechanisms interact and distribute a common apoptosis final phase that destroys substrates necessary for cell maintenance.[23]

While intracellular signals that directly act on targets are mitochondrial-dependent actions, the intrinsic pathway is independent of receptor involvement.[24] Any stimulus that does not result in the repression of death processes, such as the absence of specific growth factors, cytokines, or hormones, is characterised as a negative signal. It might start with positive or negative stimuli. Positive stimuli include things like radiation, hypoxia, toxins, hyperthermia, and other conditions that might cause apoptosis.[25] The loss of mitochondrial transmembrane potential results from modifications in the permeability transition in the mitochondria. Pro-apoptotic proteins are transported from the intermembrane space of the mitochondria to the cytosol, including Smac/ DIABLO, cytochrome c, and the serine protease (as the first group) and endonuclease G, HtrA2/ Omi AIF, and caspase-activated DNase (as the second group).[26]

The caspase-dependent mitochondrial pathway is activated by the first group. According to this group, cytochrome c binds to and activates Apaf-1, pro-caspase 9, and Smac/ DIABLO to form "apoptosome," while HtrA2/ Omi encourages apoptosis by blocking IAP (inhibitors of apoptosis protein) activity.[27] The main effect of pro-apoptotic protein release is caspase 9 activation.[28] During apoptosis, the second group of pro-apoptotic proteins is released from the mitochondria. Caspase-independent proteins that go to the nucleus and fragment DNA include AIF and endonuclease G.[29] In continuation with the apoptotic studies, docking studies were carried out to investigate the docking potential of two selected GC-MS spectral phytocompounds *viz.*, squalene and rhodoxanthin present in *H. undatus* against AIF, Apaf-1, BAK, caspase 8 and RIP proteins.

In our docking results, the ligand squalene when docked with AIF, Apaf-1, BAK, caspase 8 and RIP showed no interaction. Likewise, rhodoxanthin also did not show any interaction with RIP protein. On the other hand, rhodoxanthin with AIF showed a GSCS for 6248 and had 2 hydrogen bonds, with Apaf-1, it showed a GSCS for 5532 and had 1 hydrogen bond, with BAK, it showed a GSCS for 6088 and had 1 hydrogen bond, and with caspase 8, it showed a GSCS for 6710 and had 1 hydrogen bond. Similar docking reports of stearic acid present in *Cardiospermum halicacabum* with HepG-2 cell line proteins such as plasminogen and transferrin,[30] GC-MS spectral compounds in propolis against caspase 3, caspase 9 and β-actin,[31] Rutin compound against TNF, caspase 3, NF-Kappa-B, p53, collagenase, nitric oxide synthase and cytochrome c,[32] and alginic acid and fucoidan substances found in *Sargasum wightii* against caspase 3, caspase 9, and β-actin [33] have been reported. Likewise, the anti-apoptotic properties of ascorbic acid, betalain, and gallic acid present in dragon fruit against caspase-3, caspase-9 and β -actin[34], pure propolis compound against

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caspase 3, caspase 9, bax, Bcl-2, and BclxL,[35] and ethyl(2s)-2-methylbutanoate and 1-(ethylsulfanyl) ethane-1-thiol present in durian fruit against caspase 3, caspase 9, bax, bcl-2 and bcl-xl,[36] isolated compounds from kiwi fruit with caspase 3 and β-Actin[37] and α, β and γ mangostin compounds from *Garcinia mangostana* fruit against caspase-3, cyclin-D and p53 have been reported[38], which supports the results of the present investigation.

Similar to this, doxorubicin (a common medication) and the seven beneficial substances from *Solanum xanthocarpum* were docked against GLUT4. When compared to several of the phytochemicals, stigmasterol glucoside showed a higher binding affinity to GLUT4 in the docking poses and binding affinity study. In comparison to caffeic acid (3176), apigenin (4146), scopoletin (3540), esculin (4772), lupeol (6284), and solasodine, PatchDock revealed a geometric form complementarity score for doxorubicin of 6600. Interestingly, stigmasterol glucoside had a binding affinity score of 7590, which was higher than that of the control (doxorubicin), and had a stronger affinity for GLUT4 than doxorubicin (6600).[39] Likewise, the docking interaction of three compounds from *Annona muricata* with apoptotic proteins as GSCS was predicted for Muricin J with caspase 3 (5096), Muricin K with caspase 3 (5068), Muricin L with caspase 3 (5348); Muricin J with caspase 9 (5130), Muricin K with caspase 9 (5474), Muricin L with caspase 9 (No Interaction); Muricin J with β-Actin (No Interaction), Muricin K with β-Actin (5646), Muricin L with β-Actin (5706).[40] These findings support the present work.

Enhancing the drug research and development process is the goal of the evaluation of drug-likeness properties. The topological polar surface area (TPSA) and molecular weight of the drug molecule affect its permeability through the biological barrier. Lower molecular weights and TPSA improve permeability while higher molecular weights decrease permeability. In terms of lipophilicity, LogP is defined as the partition coefficient of the candidate molecule in the inorganic and aqueous phases. The drug's molecule's absorption into the body is impacted by lipophilicity. Lower absorption is related to higher LogP, and vice versa. The candidate molecule's solubility is influenced by the LogS value, and the lowest value is always preferable. A drug molecule's capacity to traverse a membrane bilayer is once more influenced by the quantity of hydrogen bond donors and acceptors that are outside of the permissible range. Oral bioavailability is affected by the increased number of rotatable bonds, and a range of 10 is considered to be appropriate.[41-43] Among the two ligands, only rhodoxanthin followed the standard rule of drug-likeness property.

Synthetic drug side effects necessitate the development of new, better medications. A biological activity demonstrates that the fruit pulp extract from *H. undatus* in methanol contains potential anti-cancer phytochemicals. In order to identify a potential lead molecule, we attempted to determine the binding affinity of specific GC-MS spectrum compounds from the methanol fruit pulp extract of *H. undatus*. Bioinformatics and computational biology have the potential to change how pharmaceuticals are designed as well as speed up the drug development process and cut expenses. The drug designing process, which uses a range of techniques to identify novel molecules, is facilitated and sped up by rational drug design (RDD). The docking of the drug molecule with the receptor is one such technique (target). The receptor is the drug action site and the one in charge of the therapeutic effect.[44]

Two secondary metabolites from *H. undatus* methanol fruit pulp extract GC-MS spectral compounds were used in this experiment to explore anticancer activity. When pharmacokinetic and pharmacodynamic factors are taken into account, rhodoxanthin is the best inhibitor for AIF, Apaf-1, BAK, and caspase 8. However, as other ligand molecules also did well in the docking experiment, they can potentially be further studied. It is hoped that this study would increase scholars' interest in new areas of research.

Conclusion

In order to identify the binding interaction of squalene and rhodoxanthin with apoptotic proteins and to correlate its docking score, structural-based docking was used in this study. The findings are in favour of creating a novel medication with enhanced liver cancer inhibitory efficacy. Based on this investigation, we draw the conclusion that rhodoxanthin from *H. undatus* is one of the finest anticancer agents. Wet lab research is still needed to confirm this possible medication candidate's precise role as an anticancer agent.

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Conflict of Interest: None

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