International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 7, Issue 6, pp: 1-6, 2022 (Special issues)
https://doi.org/10.55126/ijzab.2022.v07.i06.sp002
http://www.ijzab.com

Research Article

2-CARBAMYL-9-[BETA-D-RIBOFURANOSYL] HYPOXANTHINE FROM RAMBUTAN FRUIT AS POTENTIAL INHIBITOR OF APOPTOTIC PROTEINS: *IN SILICO* MOLECULAR DOCKING APPROACH

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Article History: Received 14th October 2022; Accepted 1st November 2022; Published 7th November 2022

ABSTRACT

The fruit of the rambutan tree, *Nephelium lappaceum*, is an important source of physiologically active substances that have the ability to induce apoptosis. Because of this, the goal of the current work was to anticipate how the GC-MS spectrum compound from *Nephelium lappaceum* (2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine]) would interact with apoptotic proteins (Caspase-3, Caspase-9, -Actin, p53, and Bcl-2) using the PatchDock docking program. As a result of the Lipinski rule, 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine is recommended as the best cancer treatment. The use of a molecule as a potential natural medicinal agent to treat disease is demonstrated through docking studies.

Keywords: Rambutan, GC-MS, Molecular Docking, PatchDock, Apoptotic Proteins.

INTRODUCTION

According to Elmore (2007), apoptosis is the pharmacodynamic endpoint of anticancer drug therapy because this occurrence ensures that there won't be any malignant resistance to chemotherapy. Furthermore, apoptosis is an autonomous process that breaks down cells into their component parts and avoids the inflammatory effect typically associated with necrosis; as a result, when cells undergo apoptosis, there is no damage to the normal surrounding cells (Elmore, 2007; Gallardo-Escarate *et al*., 2007). Deregulation of apoptotic pathways has a role in the development of conditions such hepatitis, liver cancer, toxic liver injury, and acute liver failure. Currently, there are certain glaring issues with HCC clinical therapy, including early diagnosis, a high likelihood of recurrence, and a lack of specialized care and cutting-edge medications (Wu *et al*., 2011). Extrinsic or intrinsic mechanisms can mediate apoptosis, which is necessary for preserving cellular homeostasis in the liver. The activation of death receptors on the cell surface initiates the extrinsic pathway, whereas the intrinsic pathway is started from the mitochondria (Eichhorst, 2005).

Clinical chemistry has benefited greatly from *in silico* research in recent years due to its ability to optimize screening and testing through the observation of specific bioactive compounds (Rajesh *et al*., 2016a; Mohamad Sitheek *et al*., 2020b). In addition to potentially accelerating the drug discovery process and lowering costs, computational biology and bioinformatics may also alter how medications are developed (Rajesh *et al*., 2016a; Jayameena *et al*., 2018; Jayaprakash *et al*., 2018; Kartika *et al*., 2018; Hemalatha *et al*., 2020b). Due to certain established links between some of these plants' traditional medicinal applications and biological activity, current trends in drug research are increasingly focusing on natural sources, particularly sources of plant origin. Therefore, the successful use of plant materials to treat and prevent infectious diseases over the years has continued to catch the interest of experts all over the world (Manimekalai *et al*., 2016; Rajesh *et al*., 2016b; Saranya *et al*., 2017; Hemalatha *et al*., 2020a; Mohamad Sitheek *et al*., 2020a; Rajesh *et al*., 2020 a,b, Manimekalai *et al*., 2021). Herbal medicine is the source of new drug leads for a variety of healthcare conditions as well as the synthesis of novel formulations in developing nations (Hemalatha *et al*., 2020a). Numerous bioactive and possibly anti-carcinogenic compounds, such

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as carotenes, dithiolthiones, flavonoids, indoles, isothiocyanates, phenols, folic acid, and vitamins C and E, can be found in fruits and vegetables (Hemalatha *et al*., 2020a; Manimekalai *et al*., 2021).

In Southeast Asian nations, the tropical fruit variety rambutan is commonly grown. To understand the natural advantages of rambutan fruit, along with element composition, a number of biological activities have been investigated (Angalammal *et al*., 2020). According to Angalammal *et al*. (2020) GC-MS analysis of the methanol endocarp extract of *N. lappaceum* identified 9 components. The current work tests a volatile molecule against apoptotic proteins called 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine, which was found by GC-MS from methanol fruit pulp extract from rambutan. The primary goal of the current study is to identify naturally occurring anticancer chemicals from rambutan fruit pulp by *in silico* docking experiments against apoptotic proteins to determine its propensity for apoptosis.

MATERIALS AND METHODS

In silico docking studies

It was determined through *in silico* molecular docking studies how the ligand, 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine, would interact with the proteins Caspase-3, Caspase-9, β-actin, p53, and Bcl-2.

Retrieval of protein sequence from Swiss-Prot

The Swiss-Prot database was used to retrieve the proteins for caspase-3, caspase-9, β-actin, p53, and Bcl-2. The accession numbers are P04637, P04639, P60709, P55211, and P42574.

Retrieval of protein structure from PDB

The structure of Caspase-3, Caspase-9, β-Actin, p53, and Bcl-2 were downloaded from PDBSum database and the PDB IDs are: 1CP3, 1JXQ, 3BYH, 1GZH, and 1G5J.

Retrieval of ligands from ACD/ChemSketch

2-D structure of ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine was downloaded. The 3-D structure of 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine was drawn using ACD/ChemSketch software.

Docking: PatchDock

Docking of ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine against Caspase-3, Caspase-9, β-Actin, p53, and Bcl-2 apoptotic proteins was carried out using PatchDock docking software.

Visualization of Protein using PyMol Viewer

The structures docked complex were then visualized using the PyMol Viewer software and the results were predicted.

RESULTS AND DISCUSSION

For molecular docking studies, the ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine was docked against apoptotic proteins *viz*., Caspase-3, Caspase-9, β-Actin, p53 and Bcl-2. The proteins such as Caspase-3, Caspase-9, β-Actin, p53 and Bcl-2 were downloaded from Swiss-Prot database. The 3-D structures of Caspase-3 **(**PDB ID-1CP3), Caspase-9 (PDB ID-1JXQ), β-Actin (PDB ID-3BYH), p53 (PDB ID-1GZH) and Bcl-2 (PDB ID-1G5J) were downloaded from PDB database and are given in Figure. 1. 3-D structure of 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine was drawn using ACD/ChemSketch software after downloading the 2-D structure of respective ligand (Figure 2).

Several docking software programs are available for this purpose. Drug designing and bioinformatics are largely utilized as a tool to probe the effects of medications on different molecules of the body. One such online docking program that was used in this investigation was PatchDock. The ligand and protein molecules can be docked with the help of this docking software, which also provides multiple docking options. It determines the proper docking of the ligand and protein molecule by calculating docking scores (Schneidman-Duhovny *et al*., 2005; Mashiach *et al*., 2008).

To compute the binding affinity and binding energy for the solution in kcal/mol between the ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine and the proteins Caspase 3, Caspase 9, -Actin, p53, and Bcl-2, in silico molecular docking was used in our example. This outcome demonstrated the existence of a binding site between each of the five proteins and the ligand. The hydrogen bond that formed between them confirmed that the docking was valid as well. Similar docking results were also done by Rajesh *et al*. (2016a), Flora Priyadarshini *et al*. (2018), Jayameena *et al*. (2018), Jayaprakash *et al*. (2018), Karthika *et al*. (2018), Rajini Selvaraj *et al*. (2019), Hemalatha *et al*. (2020b), Mohamad Sitheek*et al*. (2020b) and Manimekalai *et al.* (2021). In addition to *in vitro* and *in vivo* methods (Paul *et al*., 2010; Boyle *et al*., 2014), *in silico* approach has also been used to predict molecules with anticancer activity (Aruleba *et al*., 2018).

This finding demonstrates the existence of a binding site between the ligand and each of the five proteins. By creating a hydrogen bond between them, the docking is also legal. The Lipinski rule's conclusion points to the examined molecule as the top therapeutic option. Docking research supports the use of substances as prospective, all-natural medicinal agents for the treatment of disease. Overall, docking experiments showed that there is a binding site between the ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] and the receptor Apoptotic proteins such as Caspase-3, Caspase-9, -Actin, p53, and Bcl-2, as well as hypoxanthine. The Lipinski rule's conclusion points to the examined molecule as the top therapeutic option. By creating a hydrogen bond between them, the docking is also legal. The findings show that *N. lappaceum* fruit extract is a promising prospective anticancer agent for cancer therapy.

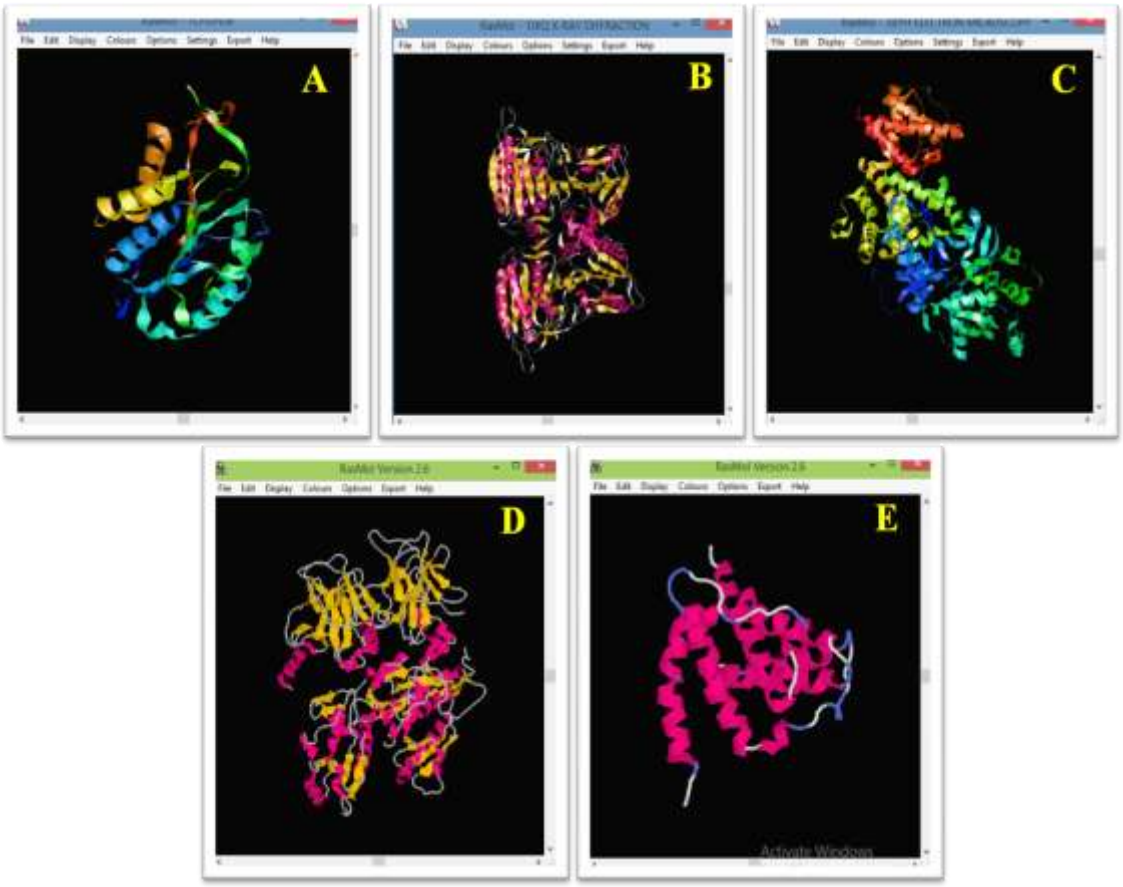

Figure 1. 3-D structure of proteins.

A. Caspase-3 B. Caspase-9 C. β-Actin D. p53 E. Bcl-2

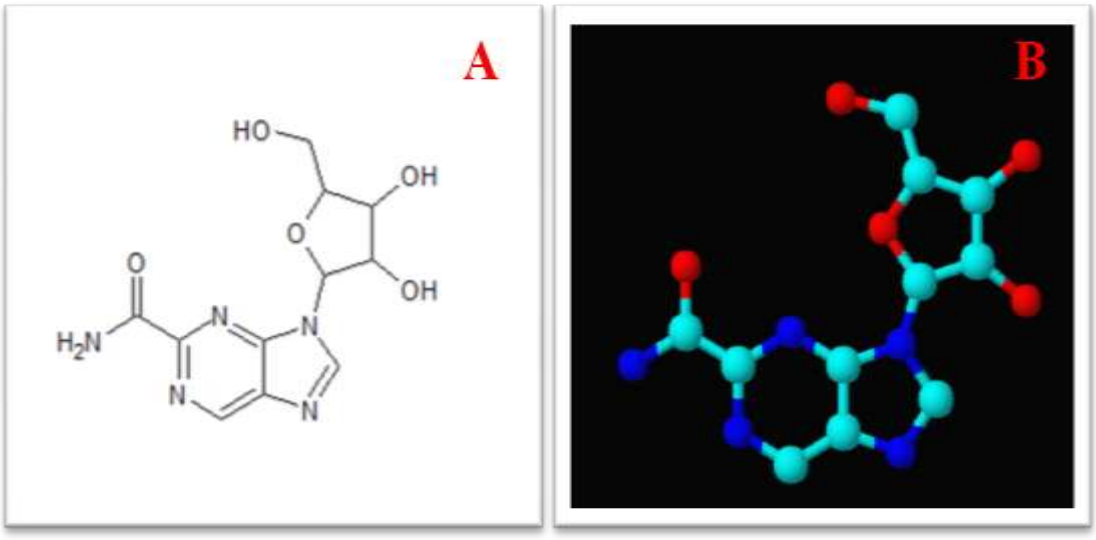

Figure 2. A. 2-D Structure of 2-Carbamyl-9-[Beta.-D-Ribofuranosyl.] Hypoxanthine

B. 3-D Structure of 2-Carbamyl-9-[Beta.-D-Ribofuranosyl.] Hypoxanthine

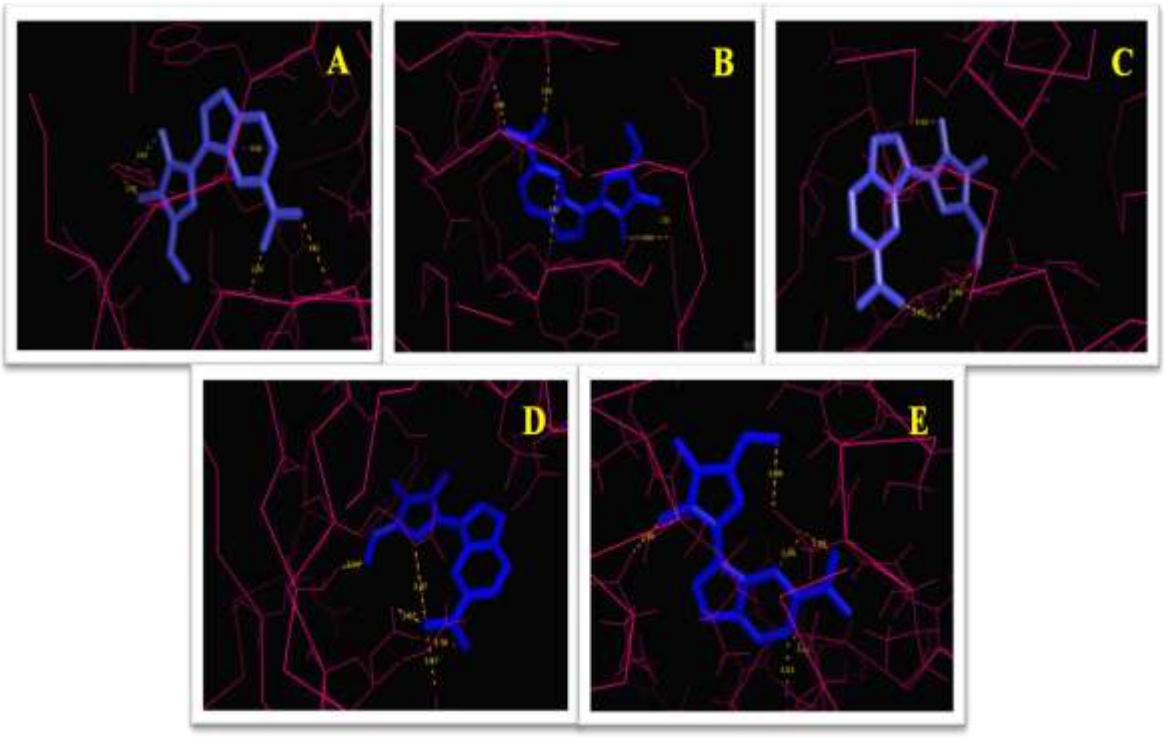

Figure 3. Visualization of docked complex with PyMol tool 2-Carbamyl-9-[Beta.-D-Ribofuranosyl.] Hypoxanthinedocked with A – Caspase-3 B - Caspase-9 C - β-Actin D - p53 E - Bcl-**2.**

Table 1. Interactions between ligand 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine with Caspase 3, Caspase 9, β-Actin, p53 and Bcl-2 proteins

Protein	Ligand	Geometrical Shape Complementarity Score	H-Bond
Caspase-3	2-Carbamyl-9-[Beta-D-	3716	5
Caspase-9	Ribofuranosyl] Hypoxanthine	3992	5
β-actin		4228	3
p53		3552	5
Bcl-2		4190	6

The 3-D structure of the inhibitor 2-Carbamyl-9-[Beta.-D-Ribofuranosyl.] Hypoxanthine against the apoptotic proteins Caspase-3, Caspase-9, β-Actin,p53 and Bcl-2were docked using Patch Dock tool. The docking results were visualized and analyzed using PyMol visualization tool (Figure 3 and Table 1). *In silico* docking study revealed the interactions between ligand and 5 proteins by *in silico* molecular docking method in order to calculate the Geometrical Shape Complementarity Score between them:

The exact mechanism should be further investigated in future studies. Also to elucidate the medicinal properties of *N. lappaceum*, there is a need for further investigation that will pave a way for finding this herbal fruit resource as a medicine to control hepatocellular carcinoma as well as for different types of cancers in future. Isolation of compounds and their assessment against HepG-2 cells to evaluate the anticancer, apoptotic and their interaction by docking studies might throw more light on the use of rambutan as an anticancer agent. Future research should look more closely at the precise mechanism. Further research is required to fully understand *N. lappaceum* therapeutic characteristics. This research will pave the path for the eventual discovery of *N. lappaceum* as a treatment for hepatocellular carcinoma as well as other cancers in the future. The usage of rambutan as an anticancer drug may be further clarified by isolating compounds and testing them against HepG-2 cells to examine the anticancer, apoptotic, and their interaction by docking studies.

CONCLUSION

Finally, molecular docking interaction of 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine GC-MS spectrum compound with apoptotic proteins results are useful for designing a novel medicine that has increased inhibitory efficacy against malignancies. Wet lab experiments will need to be conducted in the future to confirm this drug's efficacy as a cancer treatment.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the Department of Zoology, Presidency College, Chennai, Tamil Nadu, Indiafor the facilities provided to carry out this research work.

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