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A VIRTUAL SCREENING AND MOLECULAR DYNAMICS SIMULATION IN-SILICO STUDY OF ANTICANCER ACTIVITY FROM *CITRUS HYSTRIX* FRUIT EXTRACT AGAINST a-pan-BCR-ABL PROTEIN OF LEUKAEMIA

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Abstract:

Citrus hystrix, a fruit that is anticancer and a member of the rutaceae family, is easily found. Kaffir lime is used both as a physical food and as a herbal remedy for a variety of ailments, including heart disease, vertigo, and indigestion. Citrus hystrix can be utilised as a comprehensive cancer treatment. As of yet, the molecular mechanism underlying kaffir lime's anticancer activity remains unclear. The crystal structure of a-pan-BCR-ABL was obtained from the Protein Data Bank.When the leukaemia protein was molecularly docked with the active component (β-pinene, terpinen-4-ol) of citrus hystrix, the proliferation of leukaemia cells was significantly reduced. This study demonstrated the potential of kaffir lime to prevent leukaemia.This study demonstrates citrus hystrix's capacity to prevent leukaemia cancer. The study's findings offer significant new drug candidate.

KEYWORDS*: Citrus hystrix, a-pan-BCR-ABL, β-pinene and terpinen-4-ol .*

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INTRODUCTION:

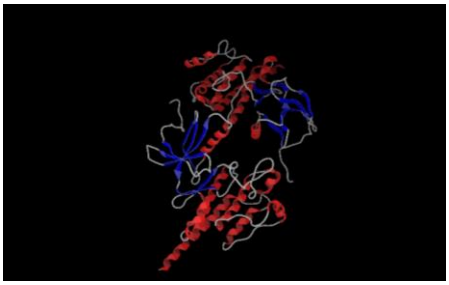
Around the world, 85% of people treat health-related problems with herbal or traditional medicine. As a result, research concerning the scientific assessment of herbal medications made from plants has advanced significantly in the last several years. Cancer, or unchecked cell proliferation, is one of the main causes of death in the world. In 2007, it claimed the lives of about 7,900,000 people worldwide, or roughly 13% of all fatalities [1].Another name for chronic myeloid leukaemia is stem cell disorder, which is caused by granulocyte cell proliferation[2]. Cultivated and widely distributed throughout many countries, especially those in Southeast Asia, is the kaffir lime (Citrus hystrix), a herbaceous plant belonging to the Rutaceae family. Kaffir lime leaves are commonly used as aromatics and as spices for flavouring because of their potent scent. According to reports, kaffir lime leaf extracts have anti-inflammatory, anti-cancer, and antioxidant properties [3].Molecular docking, also known as ligand-protein docking, is a significant computer-assisted drug design technique that is used to ascertain the affinity of recently discovered drugs with the targeted enzymes based on chromatographic results. Through a review of the literature, ligand structures and protein interactions in leukaemia pathways can be identified. The current study set out to look into citrus hystrix's in-silico activity[4].

In-silico docking studies:

Collection of materials

Protein

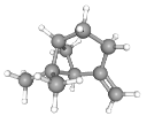
a-pan- BCR-ABL protein is collected from protein data bank.



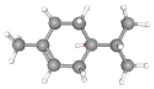
BCR-ABL Protein structure

Ligand

β-pinene, terpiene-4-ol chemical was obtained from pubchem.



β -pinene



terpinene-4-ol

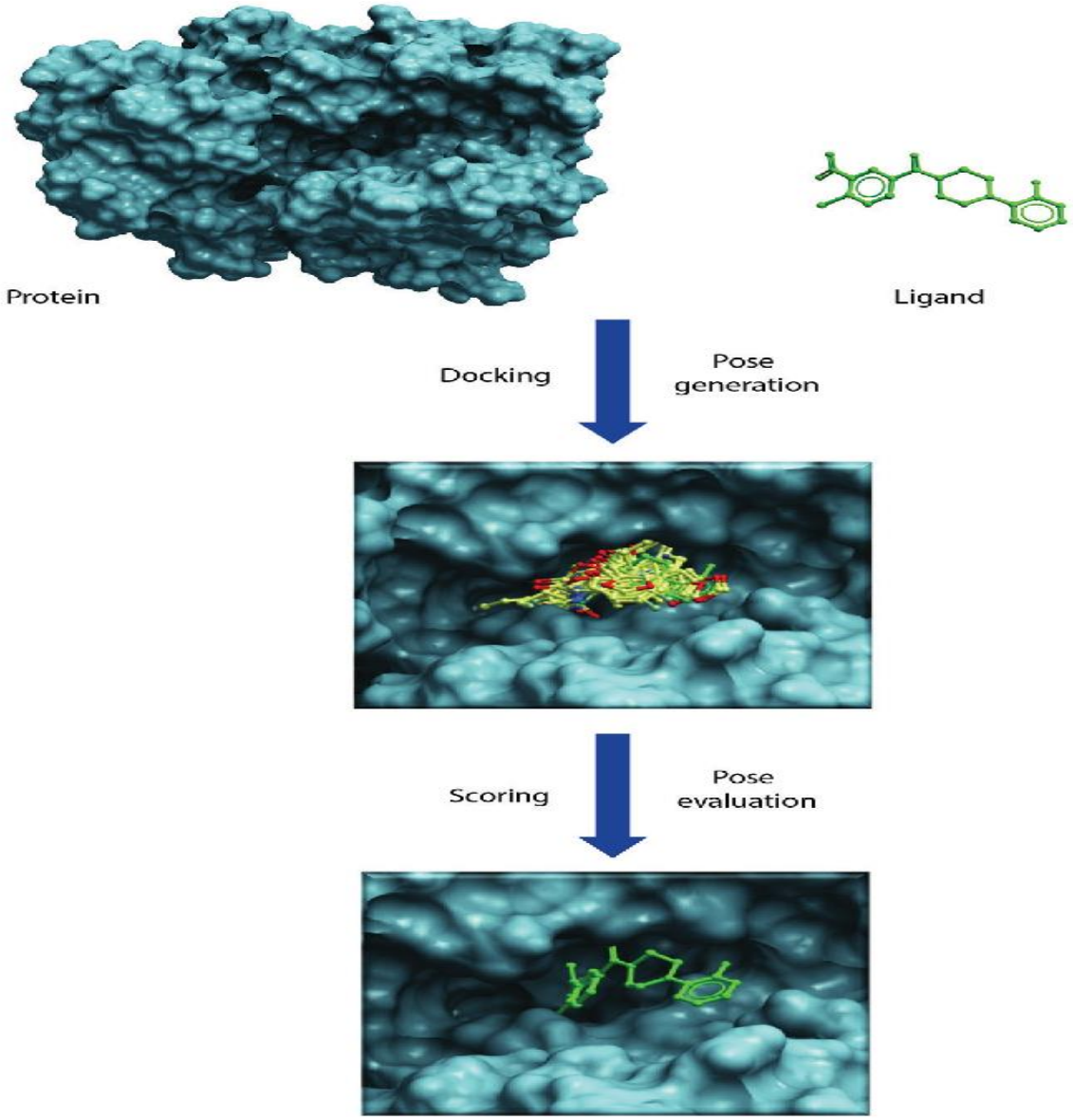


Fig 1: Representation of protein - ligand docking

A review of the literature and the compounds' activities helped choose which one to use for the docking studies. This chemical structure's ligands were selected and used to bind with targets in order to determine the binding affinity for the docking studies. The identified compounds were examined using the online docking website seamdock. The three molecules that comprise the protein sequence of the BCR-ABL are β-pinene, and terpinene-4-ol.

Disease profile:

Leukaemia is a frequent cancer that affects both adults and children. It develops when abnormalities in normal cell regulatory processes lead to the unchecked growth of hematopoietic stem cells in the bone marrow. In the US, the age-adjusted incidence of leukaemia is 12.8 per 100,000 people annually.Acute lymphoblastic, acute myelogenous, chronic lymphocytic, and chronic myelogenous are the four subtypes of leukaemia. Hematopoietic stem cell clonal proliferation in the bone marrow is known as leukaemia. While the other subtypes of lymphoblastic leukaemia are more common in adults, lymphoblastic leukaemia is more common in children.. Genetic predispositions and environmental factors, such as exposure to ionising radiation, are examples of risk factors. The non-specific symptoms include fever, exhaustion, loss of weight, pain in the bones, bruises, or bleeding. Leukocytosis and other abnormally elevated or depressed cell lines are typically revealed by a complete blood count.

Referrals to hematologist-oncologists should be made as soon as possible for patients with suspected leukaemia. Additional testing on peripheral blood or bone marrow confirms the diagnosis. Chemotherapy, radiation therapy, monoclonal antibodies, and hematopoietic stem cell transplantation are possible forms of treatment. Tumour lysis syndrome and major infections resulting from immunosuppression are among the treatment's complications. Survivors of leukaemia should be closely watched for cardiac problems, metastatic cancers, and endocrine disorders such hypothyroidism, hypogonadism, and metabolic syndrome. Younger patients and those with chronic lymphocytic or myelogenous leukaemia have the highest five-year survival rates[4].

Figure 2: Represtation of Leukemia and symptoms of leukemia.

BCR-ABL protein of leukaemia :

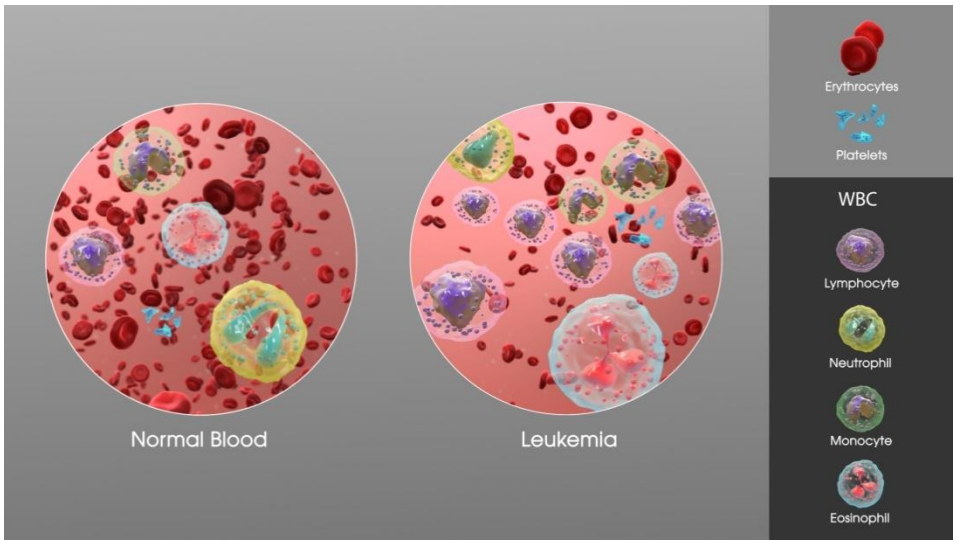


Figure 3 : Re-presentation of leukemia

A clonal expansion of at least 5,000 B lymphocytes per μL (5.0 × 109 per L) in peripheral blood, as verified by immunophenotyping, is the basis for the diagnosis of chronic lymphocytic leukaemia. Although it is not necessary to obtain a bone marrow specimen for the diagnosis of chronic lymphocytic leukaemia, it can be obtained to ascertain the degree of marrow involvement for prognostic purposes[5]. Testing for a specific abnormality known as the Philadelphia chromosome, or the BCR-ABL1 fusion gene, in bone marrow or peripheral blood is necessary for the diagnosis of chronic myelogenous leukemia[6]. A reciprocal translocation between chromosomes 9 and 22 causes the BCR-ABL1 fusion gene to form in chronic myelogenous leukaemia, which interferes with the bone marrow's regular cell regulatory functions. Ninety-five percent of patients with chronic myelogenous leukaemia have shortened chromosome 22, also known as the Philadelphia chromosome [7].Although the chromosomal rearrangement in the remaining 5% of patients is different, the aberrant BCR-ABL1 fusion gene is still formed.

DIAGNOSIS:

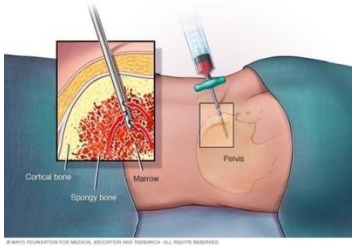


Figure 4 : Represtation of diagnosis of leukemia

A complete blood count should be done if leukaemia is suspected. In chronic myelogenous leukaemia and chronic lymphocytic leukaemia, marked leukocytosis—often more than 100,000 white blood cells per μL (100.0 × 109 per L)—is the hallmark laboratory finding. Compared to only 34% to 38% of patients with acute myelogenous leukaemia or acute lymphoblastic leukaemia, over 96% of patients with chronic myelogenous leukaemia have white blood cell counts greater than 20,000 per μL (20.0 × 109 per L). Leukopenia may also coexist with thrombocytopenia or anaemia in cases of acute leukaemia. Initial laboratory tests that can be useful include coagulation studies, liver function tests, and the measurement of serum electrolyte and creatinine levels. When a patient exhibits symptoms of illness or fever, the doctor should perform urinalysis, urine culture, blood culture, and chest radiography to assess for infection. A peripheral blood smear and typically a bone marrow specimen (an aspirate or core biopsy) are required for the next stage of the diagnosis[11][5].

Plant profile:

Botanical name	*Citrus hystrix*
Synonym	Citrus
Family	Rutaceae
English name	Kaffir lime
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Sapindales
Species	*Citrus hystrix*

Figure 5 : *CITRUS HYSTRIX*

Table 1 : Chemical constituents

Source	Active chemical constituents	Nature of extract
Leaf	Beta-pinene, sabinene, D-limonene, beta-citronallal, terpinene-4-ol, alpha-terpinene.	Methanolic extract, ethanolic extracts, water, fraction.

MATERIALS AND METHODS:

Materials:

For our current study, we used biological databases like PubChem, seamdock, and PDB (Protein Data Bank), as well as software like autodock vina.

PDB (Protein Data Bank):

Established in 1971 at Brookhaven National Laboratories (BNL), the Protein Data Bank (PDB) is the only global database for structural data on biological macromolecules**.**

PubChem:

PubChem, a public database, provides information on chemicals and their biological roles (https://pubchem.ncbi.nlm.nih.gov).

As part of the US National Institutes of Health (NIH) Molecular Libraries Roadmap Initiatives, Pub Chem was initially introduced in 2004. Since then, it has expanded to become a significant chemical data repository that serves the scientific communities in a variety of fields, including drug discovery, medicinal chemistry, cheminformatics, and chemical biology.

Over the past eleven years, PubChem has expanded into a substantial infrastructure that provides the scientific research community with access to chemical information. The three interconnected databases that make up PubChem are substance, compound, and bioassay.

Individual PubChem data contributors have contributed chemical data to the Substance database, and specific chemical structures have been taken out of the Substance database for the Compound database. The Bio Assay database contains details on the biological activity of chemical substances that have been examined in assay tests.

An overview of the PubChem Substance and Compound databases is given in this document, along with details on their contents, organisation, data sources, and standardisation of chemical structures. Additionally, it has programmatic access and web-based interfaces for both text and non-text searches.

It provides a brief synopsis of PubChem RDF as well as PubChem3D, a resource constructed from theoretically precise three-dimensional models of molecules in PubChem, which are formatted versions of the data that can be used for data sharing, analysis, and integration with data from other databases.

Autodockvina :

AUTODOCK VINA is a virtual screening tool for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. Using Autodock Vina, medicinal chemists can perform virtual screening. The software helps users at every step of the way, from data preparation to grid box viewing. The Autodock wizard is a helpful tool for logical drug design, even though it lacks any magic buttons that can be used to discover new medications. Its chemical spreads heat-like functionality and intuitive interface help.

The Autodock Vina app will be installed by the application.

SEAMDOCK :

In silico assessment of protein receptor interactions with small ligands is now a standard part of drug discovery pipelines, and a multitude of tools and protocols have been developed to that purpose. In order to facilitate both local and/or global docking of ligands as well as a hierarchical method that combines the two for straightforward interaction site identification, the online SeamDock service unifies different docking tools into a single framework. There is no need to install any additional software in order to use this service; all you need is a standard web browser. The seamless library links the user's webpage to the RPBS calculation server so that the user can easily and interactively navigate the SeamDock website. The visualisation of ligand, receptor, and docking poses in three dimensions, as well as their interactions with the receptor, has been the subject of extensive research. The advanced visualisation features and the seamless library allow a user to share a docking session and all of its visualisation states with an infinite number of collaborators. For this reason, SeamDock is a dynamic, free, easy-to-use, educational online docking tool that works best for training and instruction.

METHODOLOGY:

Docking procedure:

Step1: Preparation of receptor protein:

Protein Data Bank (PDB) provided the 3D crystallographic structure of the protein a-pan-BCR-

ABL (PDB ID:3ik3). The protein's three-dimensional structure was found. Open the file and read the molecule.

- Molegro Molecular Viewer → Import file → Export molecule.
- Protein is only applied → Export → Save as pdb file.
- Save as prepared protein (PDB form).

Step2: Preparation of ligand:

β -pinene and terpinene-4-ol is taken as ligand molecule. They are downloaded from pubchem and then converted into PDB format.

- Molegro Molecular Viewer → Import → File → Export molecule.
- Save as (PDB form)

Step3: Docking:

AUTODOCK:

- File → Read molecule → Select the protein structure.
- Click on edit → Hydrogens → Add polar only.
- click on edit→ Charges → Add compute charges.
- Click on ligand → Choose ligand → Select the ligand structure.
- Click on edit →Charges → Add kollamen charges.
- Click on grid → Select the grid box → Select the dimensions of your grid box.
- Note your grid dimensions.

Step 4: Docking:

- By using https;//bioserv.rpbs.univ-paris-diderot.fr/services/seamdock
- Click on run seamdock →Choose ligand (in the .PDB, .SDF form) → click on open.
- Click on choose protein →Choose protein (in the form) → click on open.
- Set the dimensions →Adujusting x,y,z → Launch Docking.

Finally 2D interactions are predicted from MOLEGRO molecular viewer.

RESULT AND DISCUSSION:

Docking result:

- The 3D structure of BCR-ABL (PDB ID:3ik3) ,with a resolution of 1.90Å respectively is obtained from the **protein data bank (RCSB-PDB).**
- The structure of the beta-pinene (CID_14896),terpinene-4-ol (CID_11230), was obtained from **Pubchem** .
- The energy minimization of the ligands was performed using **Autodock vina**.
- **Seamdock** is used to estimate the affinities and interactions of protein and ligand.

Table 2 : Binding affinities

Ligand	Protein target	Binding Affinity
β –pinene	a pan-BCR-ABL protein	-6.8 kcl/mol
Terpinene-4-ol		-6.1 kcl/mol

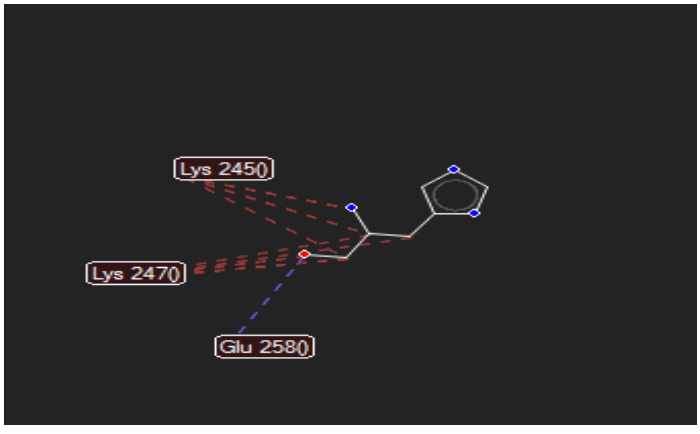


Figure 6 : 2D interaction of β -pinene with a pan-BCR-ABL protein

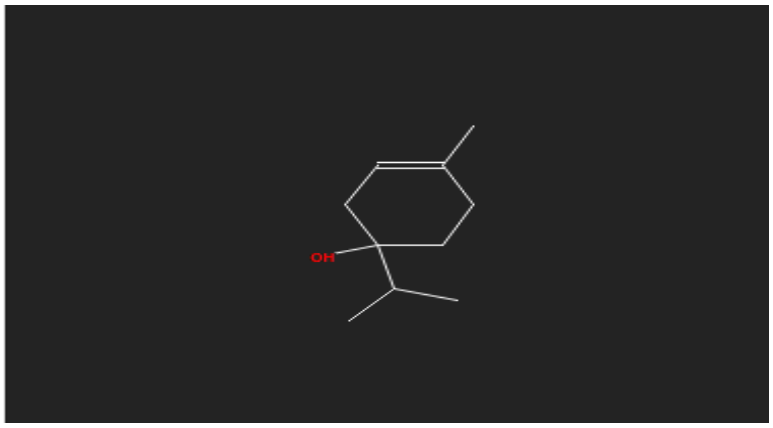


Figure 7 : 2D interaction of terpinene-4-ol with a pan-BCR-ABL protein

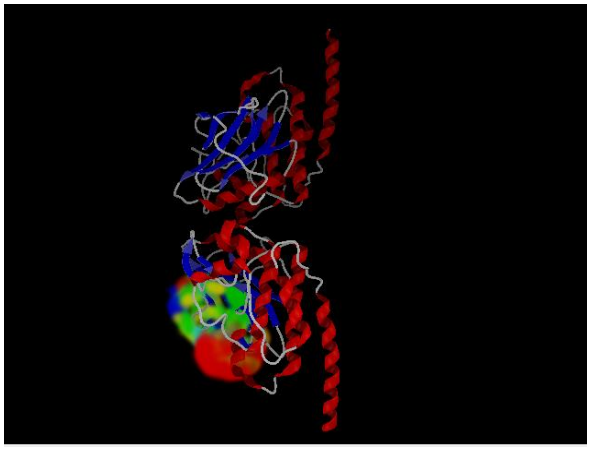


Figure 8: 3D representation with energy mapping of interaction β -pinene with a pan-BCR-ABL protein

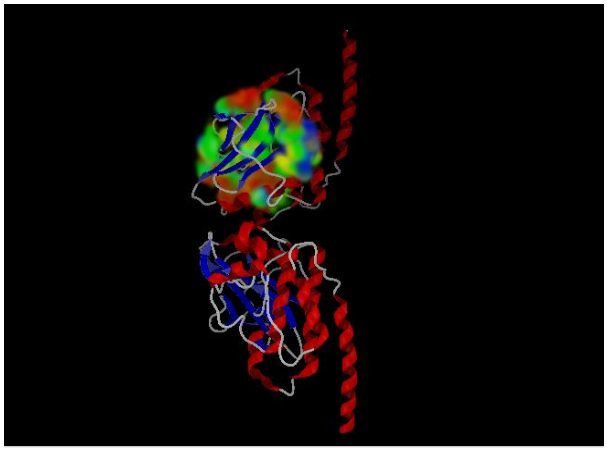


Figure 9: 3D representation with energy mapping of terpinene-4-ol with a pan-BCR-ABL protein

DISCUSSION:

- Himansu et al. "In-silico identification of inhibitors against mutated protein of chronic myeloid leukemia: a virtual screening and molecular dynamics simulation study" by targeting protein was taken……
- According to Fajarina, Sudewi et al. anti-cancer activity of *citrus hystrix* leaf extract against leukemia was taken for present docking studies.
- According to kumar *et al.* BCR-ABL is the causatives for leukemia cancer was taken for the present docking studies.
- The protein a-pan-BCR-ABL was docked with β -pinene and terpinene-4-ol using Seamdock in the current investigation.
- The anti-cancer protein ligands' binding scores were -6.8 kcal/mol and -6.1 kcal/mol.
- As a result, the protein and ligand have a stronger binding affinity when the docking score is least negative. The binding energy of beta-pinene with a-pan-BCR-ABL protein was found to be -7.8 kcal/mol.

CONCLUSION:

- The outcomes of the insilico research provided a useful deduction regarding the compounds' usage, demonstrating increased binding energy and affinity towards enzymes and being utilised as leukemia treatment aids..
- In the current investigation, the target protein a-pan-BCR-ABL was successfully docked with active constituents (Beta-pinene and terpinene-4-ol). β -pinene's binding energy was recored at -6.8 kcal/mol.
- Because the protein and ligand have a stronger binding affinity when there is less negative binding score, this could potentially be a medication with anti-cancer activity.

REFERENCES:

1. Donna S. Shewach Guest Editor, University of Michigan School of Medicine Robert D. Kuchta Issue Editor, University of Colorado, Boulder CR900208X.
2. Chronic Myeloid Leukemia is known as stem cell disorder resulted by proliferative or excessive division of granulocyte cells.
3. Antileukemic Cell Proliferation of Active Compounds from Kaffir Lime (Citrus hystrix) Leaves.
4. *In Silico* Anticancer Evaluation, Molecular Docking and Pharmacophore Modeling of Flavonoids against Various Cancer Targets**Author(s):** Jainey Puthenveettil James*, Pankaj Kumar*, Abhishek Kumar, Katte Ishwar Bhat and Chakrakodi Shashidhara ShastryVolume 17, Issue 12, 2020 **Page:** [1485-1501]**Pages:** 17**DOI:** 10.2174/1570180817999200730164222
5. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines [published correction appears in *Blood.* 2008;112(13):5259]. *Blood.* 2008;111(12):5446-5456.
6. Davis, Amanda S et al. "Leukemia: an overview for primary care." *American family physician* vol. 89,9 (2014): 731-8.
7. Sawyers CL. Chronic myeloid leukemia. *N Engl J Med.* 1999;340(17):1330-1340.
8. Moen MD, McKeage K, Plosker GL, Siddiqui MA. Imatinib: a review of its use in chr
9. Sinigaglia R, Gigante C, Bisinella G, Varotto S, Zanesco L, Turra S. Musculoskeletal manifestations in pediatric acute leukemia. *J Pediatr Orthop.* 2008;28(1):20-28.
10. Ma SK, Chan GC, Ha SY, Chiu DC, Lau YL, Chan LC. Clinical presentation, hematologic features and treatment outcome of childhood acute lymphoblastic leukemia: a review of 73 cases in Hong Kong. *Hematol Oncol.* 1997;15(3):141-149.
11. Savage DG, Szydlo RM, Goldman JM. Clinical features at diagnosis in 430 patients with chronic myeloid leukaemia seen at a referral centre over a 16-year period. *Br J Haematol.* 1997;96(1):111-116
12. National Comprehensive Cancer Network. Clinical practice guidelines in oncology: acute myeloid leukemia. http://www.nccn.org/professionals/physician_gls/pdf/aml.pdf (subscription required). Accessed January 9, 2014.
13. Puji Kurniawati Rahman, Hamiseh, & Teguh Setiawan Wibowo. (2023). Pharmacological Activities of Citrus hystrix. Indonesian Journal of Interdisciplinary Research in Science and Technology, 1(7), 641–650. https://doi.org/10.55927/marcopolo.v1i7.5813
14. Siti HN, Mohamed S, Kamisah Y. Potential Therapeutic Effects of *Citrus hystrix* DC and Its Bioactive Compounds on Metabolic Disorders. Pharmaceuticals (Basel). 2022 Jan 29;15(2):167. doi: 10.3390/ph15020167. PMID: 35215280; PMCID: PMC8875002.
15. Ho Y, Suphrom N, Daowtak K, Potup P, Thongsri Y, Usuwanthim K. Anticancer Effect

of *Citrus hystrix* DC. Leaf Extract and Its Bioactive Constituents Citronellol and, Citronellal on the Triple Negative Breast Cancer MDA-MB-231 Cell Line. Pharmaceuticals (Basel). 2020 Dec 18;13(12):476. doi: 10.3390/ph13120476. PMID: 33353129; PMCID: PMC7766836.

16. Tunjung, Woro Anindito Sri, et al. "Evaluation of Anticancer Bioactive Compounds and Cytotoxicity of Kaffir Lime (Citrus hystrix Dc.) Callus Extract Post Preservation." Indonesian Journal of Pharmacy (2021): 179-192.
17. Maher T, Ahmad Raus R, Daddiouaissa D, Ahmad F, Adzhar NS, Latif ES, Abdulhafiz F, Mohammed A. Medicinal Plants with Anti-Leukemic Effects: A Review. Molecules. 2021 May 7;26(9):2741. doi: 10.3390/molecules26092741. PMID: 34066963; PMCID: PMC8124366.
18. Ogochukwu Izuegbuna (2022) Leukemia Chemoprevention and Therapeutic Potentials: Selected Medicinal Plants with Anti-Leukemic Activities, Nutrition and Cancer, 74:2, 437-449, DOI: 10.1080/01635581.2021.1924209
19. Bergmann, Lothar, Ulrich Maurer, and Eckhart Weidmann. "Wilms tumor gene expression in acute myeloid leukemias." *Leukemia & lymphoma* 25.5-6 (1997): 435-443.
20. Ampasavate, Chadarat, Siriporn Okonogi, and Songyot Anuchapreeda. "Cytotoxicity of extracts from fruit plants against leukemic cell lines." *Afr. J. Pharm. Pharmacol* 4.1 (2010): 13-21.
21. Chueahongthong, Fah, et al. "Cytotoxic effects of crude kaffir lime (Citrus hystrix, DC.) leaf fractional extracts on leukemic cell lines." *J Med Plants Res* 5.14 (2011): 3097-3105.
22. Lawrence, Brian M., et al. "Constituents of the leaf and peel oils of Citrus hystrix, DC." *Phytochemistry* 10.6 (1971): 1404-1405.
23. SUGIYAMA, Satoko, et al. "Studies on the differentiation inducers of myeloid leukemic cells from Citrus species." *Chemical and pharmaceutical bulletin* 41.4 (1993): 714-719.
24. Deshpande, Sanjay H et al. “In Silico Study on the Interactions, Molecular Docking, Dynamics and Simulation of Potential Compounds from *Withania somnifera* (L.) Dunal Root against Cancer by Targeting KAT6A.” *Molecules (Basel, Switzerland)* vol. 28,3 1117. 22 Jan. 2023, doi:10.3390/molecules28031117
25. Kumar, Himansu et al. “In-silico identification of inhibitors against mutated BCR-ABL protein of chronic myeloid leukemia: a virtual screening and molecular dynamics simulation study.” Journal of biomolecular structure & dynamics vol. 34,10 (2016): 2171-83. doi:10.1080/07391102.2015.1110046