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Green leafy Vegetables: An overview of In-silico studies of Anti-cancer activity

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ABSTRACT:
Green leafy vegetables are recognized for their rich content of bioactive compounds that exhibit potential health benefits, including anti-cancer properties. In recent years, computational approaches such as docking, molecular dynamics, and homology modeling have been pivotal in elucidating the molecular mechanisms through which these bioactive compounds exert their anti-cancer effects. This review provides an overview of in-silico studies focused on identifying and characterizing the bioactive constituents of green leafy vegetables, highlighting their interactions with key cancer-related molecular targets such as enzymes, receptors, and signaling pathways. Specific phytochemicals, including flavonoids, carotenoids, and glucosinolates, have been identified through computational analyses for their ability to modulate cancer cell proliferation, apoptosis, and metastasis. The integration of computational and experimental approaches not only enhances our understanding of the molecular basis of anti-cancer activities but also holds promise for the development of novel therapeutic strategies derived from natural sources. Further advancements in computational methodologies and validation through preclinical and clinical studies are essential for translating these findings into effective cancer prevention and treatment modalities.
Amaranthus Tricolour
S. Pulipati, P. Srinivasa Babu and H. Dommati
Isolated and characterized and evaluate anticancer activity of a lead phytochemical from leaves of Amaranthus tricolor (L)

I. INTRODUCTION:

These days, cancer is a major concern. The direction of healthcare research that is trending is the development of emotional and side effects-lacking anticancer therapies. The purpose of this work is to extract, identify, and assess a lead phytochemical from Amaranthus tricolor (L) leaves for its potential anticancer properties. A. tricolor (L) leaf methanolic extract was made by cold maceration and designated as ATME. Chloroform and water were separated in the same volume for the ATME extract. The HPTLC recommended mobile phase of n-hexane:ethyl acetate (6:4 v/v) was used to further fractionate the chloroform extract.

Cancer is a large group of diseases that can start in almost any organ or tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body and/or spread to other organs. It is one of the major leading causes of death.According to this study we tried to isolate an anticancer bioactive principle from leaves of Amaranthus tricolor (L), and the extraction of amaranthustricolour was done.

Extraction of Amaranthus Tricolour:The powdered plant material of A.tricolor was extracted by cold maceration process. The powder of 1kg each plant material was imbibed with 1 L of methanol and incubated on shaker for 3 days.The extract was filtered using muslin cloth and the process was repeated twice. The percentage yield of extract was calculated and preserved in desiccator for further study

Fractionation of Amaranthus Tricolour**:**2 g of ATME was dissolved by adding 100 mL of chloroform. The extract was taken in separating funnel and mixed with equal volume of water. The separating funnel wasshaken thoroughly to separate chloroform and aqueous fractions. The fraction was subjected to HPTLC to optimize mobile phase.

Fingerprint analysis of chloroform fraction of ATME by HPTLC:Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 350 nm operated by WINCATS software version 1.4.2. After that Bioautographic screening was performed. The fractions of extract with good sensitivity against microorganisms were determined by contact bioautography. The chromatogram was placed onto the inoculated agar medium facing down and allowed to diffuse the fractions for 3-4 hrs. Among these the third fraction (SOWIS-III) was selected for further study because of higher inhibition rate of bacteria.

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Isolation of Phytoconstituents: The separation and isolation of chloroform fraction of ATME was done by column chromatography as per the HPTLC compounds present in CHCl3 fraction of ATME. Wet packing mount method was used to enhanced by mixing extract with silica gel G (Mesh 60-120). The separation and isolation of phytoconstituents was done with the help of mobile phase nhexane: ethyl acetate (6:4 v/v). hence fractions were pooled together and further purified by preparative TLC and confirmed by HPLC

Structural Interpretation of Isolated Compounds:According to bioautography, fractions I, III, V, VI and VIII may act as antibacterial agents. Among these the III fraction, SOWIS-III was further studied for structural interpretation, in silico analysis and biological activities evaluation. The purity of the fraction was confirmed byHPLC and the structure waselucidated by NMR and Mass spectroscopical studies. The compound was identified as a flavonol glycoside 24-Methylene cycloartenol

Insilico analysis of AmranthusTricolour:The physiological effects of these steroids are mediated by a ligand-inducible nuclear transcription factor, the oestrogen receptor (ER). Oestrogens are involved in the growth, development and homeostasis of a number of tissues. The expression of Oestrogens is elevated in many cancers. Hence, it was taken as attractive drug target protein for docking study of 24-Methylene cycloartanol.

In Methodology of ligand preparation the ligands were initially drawn using chemsketch tool and is converted into protein data bank format for the docking.

In Protein preparation: The human oestrogen receptor was prepared with auto dock 4.02v software.

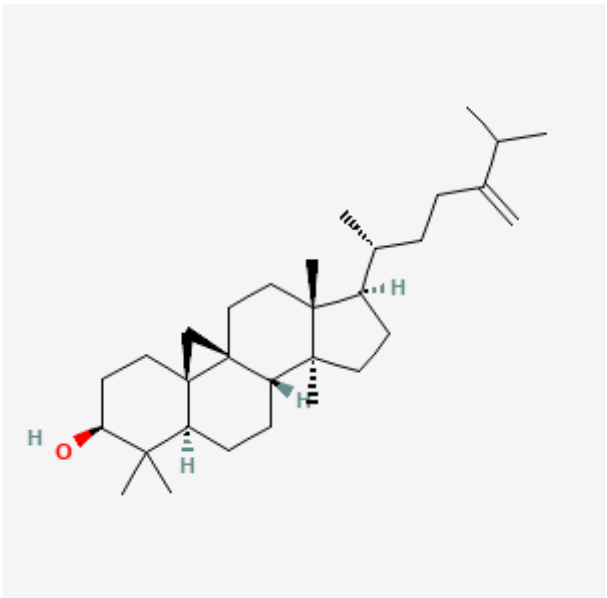

Fig 1: Structure of 24-Methylene cycloartenol

Molecular docking: Human oestrogen receptor ligandbinding domain of PDB:1ERE was chosen and docked with 24-Methylene cycloartanol.

II. RESULT:

The phytochemical screening of ATME revealed that the presence of various constituents such as carbohydrates, amino acids, proteins, alkaloids, glycosides, steroids, flavonoids, tannins and phenolic compounds. The presence of these secondary metabolites lead to further study on In silico analysis, fractionation, isolation, structural elucidation and cytotoxic activity evaluation of ATME. The ATME was further fractionated into chloroform andthe HPTLC fingerprint analysis of chloroform fraction of ATME suggested the presence of 11 compounds.

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H .Akasaka , Y.Mizushina , K.Yoshida , Y. Ejima , N. Mukumoto , T. Wang , S.Inubushi , M.Nakayama1 , Y.Wakahara and R. Sasaki demonstrated that MGDG enhances the cytotoxicity of radiation to induce apoptosis of cancer cells in vitro and in vivo.

Pancreatic cancer remains a major public health issue as the third leading cause of cancer-related death in Europe.Recent in vitro and in vivo studies have shown that consumption of vegetables and fruits with chemopreventive components can reduce cancer risk.The chloroplast thylakoid membrane of higher plants contains glycoglycerolipids such as monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) ; these compounds have potential anti-cancer functions includinginhibition of DNA polymerase and suppression of cancer cell proliferation, with MGDG showing more potent antitumorigenic and anti-inflammatory activity than the others.Spinach is a major source of glycoglycerolipids and has the highest MGDG content among vegetables, fruits and grains tested to date [10–12].According to this survey, MGDG enhanced the cytotoxic effects of gemcitabine (GEM)—a key

Trigonella foenum-graecum

S.S Alghamdi, R.S. Suliman1,A.SulaimanAlsaee , K.K.Almutairi, N.A. AljammazAltolayyan aim to evaluate the anticancer activity of methanolic fenugreek extract against several cancer cell lines.

Introduction: Fenugreek, also known as Trigonella foenum-graecum L, is a natural plant that belongs to the Fabaceae family and has been known as a promising source of bioactive compounds. It has been widely used as traditional medicine since it has shown to lower blood glucose, manage cholesterol levels and further aid in the prevention and treatment of cancer.

Extractionstandard method of fenugreek extraction using the Soxhlet apparatus.Methanol was used as an extraction solvent, and a 50-mL volume of solvent was intro-duced into the extractor thimble from the top to facilitate flushing of the extractor chamber. After the extraction process has been done, the extraction was dried by rotary evaporator apparatus to get a powder form of the extraction. Further extraction has been done using other extract solvents by the maceration method. A 100 g of powder fenugreek was soaked with 1 liter of each solvent as follows: chloroform, water, diethyl ether, and methanol at 40°C for 48 h in a hot plate.The mixture was kept soaked under a magnetic stirrer hot plate at 40°C for 48 h. Then, the extract was collected and filtered using filter paper and bacterial filter.

Chemical Identification: All extracts were first injected into the Agilent 1260 Infinity HPLC system (Agilent, Germany) with Diode-Array Detection (DAD) detector. The separation carried out in a reversed-phase mode using Phenomenex Kinetex- C18 column.All extracts were first injected into the Agilent 1260 Infinity HPLC system (Agilent, Germany) with Diode-Array Detection (DAD) detector. The separation carried out in a reversed-phase mode using Phenomenex Kinetex- C18 columnAll extracts were first injected into the Agilent 1260 Infinity HPLC system with Diode- Array Detection (DAD) detector. The analysis of all extracts was performed on Agilent 1260 Infinity HPLC system.

The flavonoid-derived compounds from fenugreek metha-nolic extract were analyzed using the online service Prediction of Activity Spectra for Substances (PASS) to determine the biological probability of a substance being active (Pa) or inactive (Pi). The calculations of the absorption, distribution, metabo-lism, and elimination (ADME) properties of the fenugreek active compounds were performed using the SwissADME web server

Insilico Analysis ofTrigonella foenum-graecum

Molecular DockingThe 3D Tubulin structure was refined, minimized, and optimized with the OPLS4 force field. Unnecessary water molecules, substrates, ions, and other subunits were removed and the β-Tubulin subunit that contains the Colchicine binding site was main-tained for the docking study.A one-step docking tool was utilized to validate the dock-ing modes, and the root-mean-square deviation (RMSD) values were below 0.366 Å for both standard precision (SP) and extra precision (XP) scoring functions of GLIDE. All the compounds were docking using SP and XP scoring functions, and post-docking analysis for the docked poses wasperformed .

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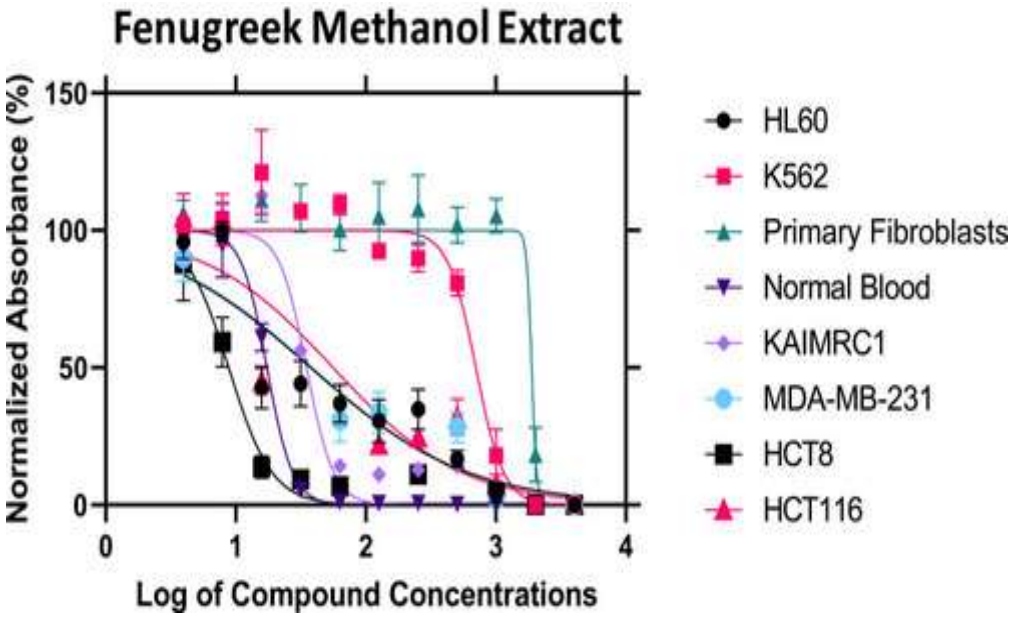

III. CONCLUSION:

This study demonstrates that the use of solvents has a significant impact on the obtained phytochemical classpresent in the fenugreek seeds. Among the four various solvents used, the phytochemical class obtained with maceration using chloroform is steroidal skeleton dios-genin, while methanol solvent contained the flavonoids.Furthermore, the computational activity predictions and in-vitro anticancer results confirmed the promising antic-ancer activity of flavonoids against several cancer cell lines that can potentially mediate through inhibition of tubulin polymerization. Thus, fenugreek methanolic extract obtained from Trigonella foenum-graecum seeds exhibits anti-tubulin activity, and further research is needed to identify new chemotherapeutic agents with less harmful side effects.

Spinaceaolarecea

S. Abdelgawad& M. Hetta1 & M. Ibrahim & P. Balachandran & J. Zhang & M. Wang & G. Fawzy & S. Ross , isolated antileukemic phytoconstituents, as well as seventeen known compounds from S.Oleracea leaves.

Introduction: Chronic myeloid leukemia (CML) is ranked as the fourth predominant cancer in upper Egypt that constituted about 10.2% of all the reported cancer cases, after breast, liver, and bladder cancers

In Method & Material the Liquid chromatography analysis was conducted using an Agilent 1100 HPLC system. HPLC-grade methanol and water solvents were used.GC/MS analysis was performed with an Agilent 7890B gas chromatograph. Optical activity was measured using an AA-65 series automatic polarimeter

Exreaction and Isolation: The fresh leaves of Spinacia oleracea L., Amaranthaceae were taken. The shade dried leaves (1.6 kg) were ground and extracted six times with 75% ethanol at room temperature. The combined 75% hydroethanolic extract was concentrated in vacuum to afford a crude extract, that was suspended in water, and fractionated successively with hexane, dichloromethane, ethyl acetate, and n-butanol to afford fractions of 31, 2.9, 7.3, and 25.3 g, respectively. The obtained fractions were subjected to biological testing against leukemia K562 cell line. Phytochemical study of Hexane fraction of S. oleracea leaves was saponified according to the published procedure and the fatty acid methyl ester (FAME) and unsaponifiable matter (USM) were then subjected to GC/MS analysis.

Insilico analysis of S.oleracea

Molecular Docking: All docking simulations were conducted using MOE 2019 software. The receptors and the ligands were prepared using the standard structure optimization protocol of the software. The receptors are PDB IDs: 3QX3, 3QRJ, 1M17, 2SRC, and 6QS9 for topoisomerase, Abl Kinase, EGFR-tyrosine kinase, SRC kinase, and albumin, respectively. Then they were energy minimized under AMBER12: EHT force field. The active sites were set as where the co-crystalized ligand was bound. The docking was performed

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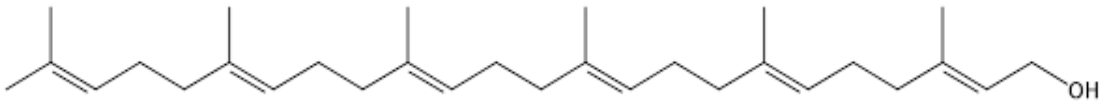
using a molecular structure of compounds isolated from S. oleracea leaves using the general protocol of MOE DOCKTITE Wizard. Triangle matcher and London dG were utilized as the placement method and scoring algorithm, respectively. The validation of docking experiments was achieved through the re-docking of the cocrystalized ligands into their corresponding active sites and then the root mean square deviation (RMSD) was calculated and docking results and score were visualized & reported.

Molecular Dynamics: To validate the retrieved binding modes from the docking study, two MD simulation experiments were conducted. The two simulation experiments were performed on the most active compound 3 in complex with Abl kinase and topoisomerase. GROMOS96 force field was implemented to generate the ligand topology using the GlycoBioChem PRODRG2 Server (Schüttelkopf and Van Aalten 2004). Later on, complex topology was generated through joining both ligand and enzymes.

Result: . The hexane, dichloromethane, ethyl acetate, n-butanol, and the aqueous fractions were tested at a concentration of 20 µg/ml and percentage inhibitions were calculated as 23, 19, 19, 20, and 18% respectively, compared to doxorubicin and Taxol (86 and 79% at 10 µM, respectively). Based on the biological screening results as well as TLC screening of the bioactive fractions, the hexane and ethyl acetate fractions were selected for further phytochemical study with the aim of isolating the bioactive compounds. Phytochemical Investigation of the Ethyl Acetate Fraction was done and the biological study were performed. The result of Molecular docking shows that a correlation between CML cell line (K562) and several target proteins whose inhibition leads to antiproliferative effect in this cell line. Four target proteins are named: human topoisomerase II beta in complex with DNA and etoposide (PDB ID–3QX3), epidermal growth factor receptor complexed with erlotinib (PDB ID–1M17), human ABL1 kinase (PDB ID–3QRJ), and SRC kinase (PDB ID–2SRC) were reported as docking targets in the K562 cell line . Molecular docking provided a mechanistic information on the possible antiproliferative activity against K562 cells through binding with Abl kinase and topoisomerase. The in silico–based safety analysis of the antileukemic compounds was tested by measuring their binding affinity to albumin (PDB ID–6QS9) The affinity of cytotoxic compounds to bind to albumin has a great impact on their pharmacodynamics and pharmacokinetic properties. Molecular dynamics (MD) simulation has been an inevitable technique in studies involving in silico drug discovery. Therefore, it was logistic to take the advantage of the MD to further endorse the docking results. Two MD simulation experiments were conducted on compound 3 bound to Abl kinase and topoisomerase. The MD results supported the docking results and highlighted the ability of the isolated compounds as antileukemic agents.

Conclusion: The hexane fraction of Spinach leaves as well as its isolated compounds, hexaprenol (1), phytol (2), and 18-[(1-oxohexadecyl) oxy]-9-octadecenoic acid shows remarkable antiproliferative activity against leukemia K562 cell line. The molecular docking study revealed that this activity is supposed to be through targeting Abl kinase and topoisomerase, and this still needs to be proved by in vitro assay of these compounds against the mentioned targets



Hexaprenol

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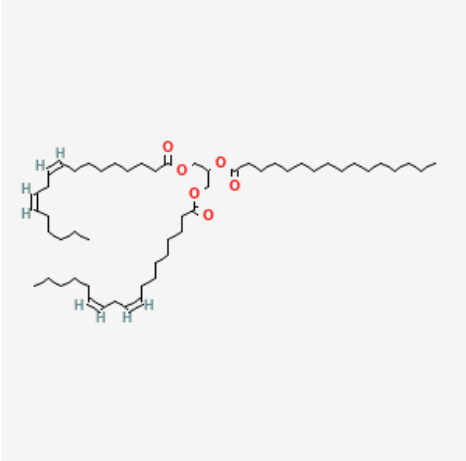
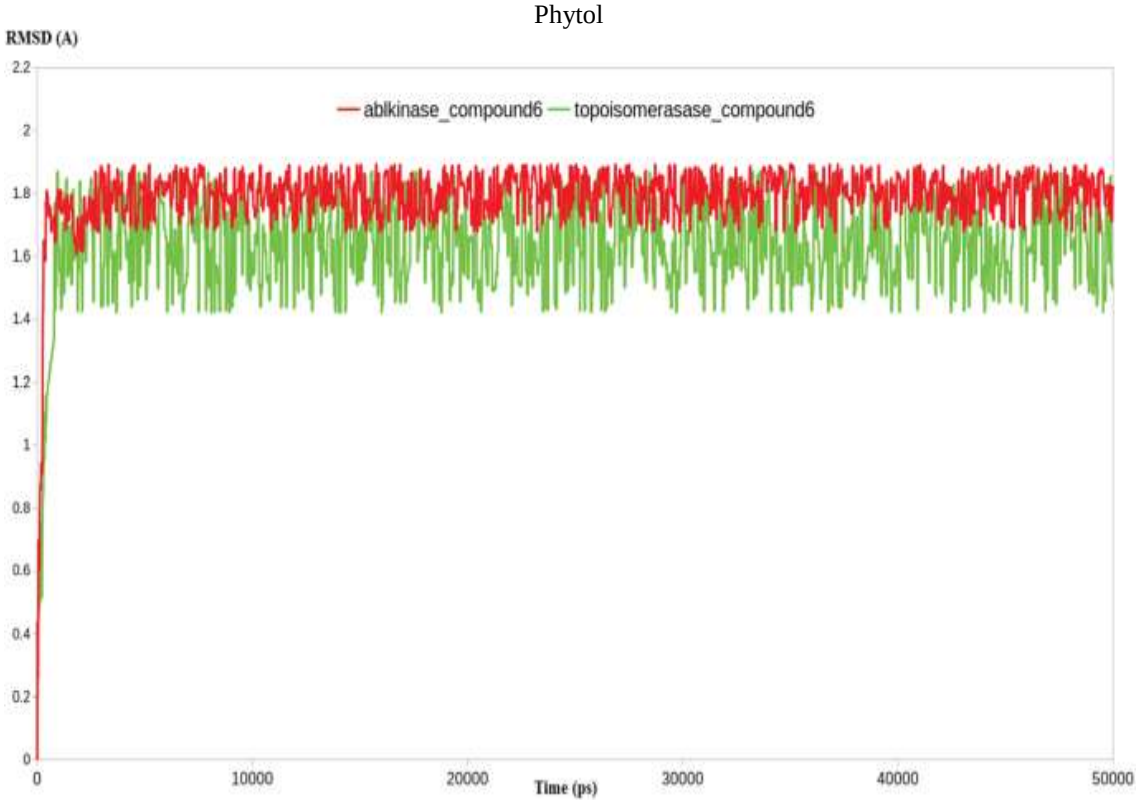


18-[(1- oxohexadecyl) oxy]-9-octadecenoic acid

Phytol



The RMSD of compound 3 with Abl kinase (red) and topoisomerase (green)

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Coriandrum sativum:

H.Machchate, R. Costa de Oliveria, I. Es-Safi, M Bouhrim, A Grafov, D.Bousta were evaluated the anticancer potential of a polyphenolic extract from C. sativum seeds against two leukemia cell lines.

Leukemia is a heterogeneous group of hematological diseases characterized by the uncontrolled and dysfunctional growth of leukocytes. They demonstrated that the polyphenol-rich fraction of the coriander seed extract contains several catechins, polyphenolic acids, and rutin. Coriander (Coriandrum sativum L.; Apiaceae) is an edible plant native to a wide area of Eurasia and Northern Africa, where it is mainly cultivated for fresh leaves and dried seeds

Extraction: The LC/MS–MS revealed the presence of nine components in the prepared extract: vanillic acid, chlorogenic acid, catechin, epicatechin, epicatechin gallate, gallocatechin, epigallocatechin, oleuropein, and rutin. Atileukemic activity: The cytotoxic activity of C. sativum polyphenolic extract was evaluated using three cell lines: human acute promyelocytic leukemia (HL60), human chronic myelogenous leukemia (K562), and normal Vero cell. The cytotoxicity indices were estimated as a cell viability percentage measured by a methylthiazoletetrazolium (MTT) assay in a dose-dependent manner. The results obtained for the CSP extract suggest a potential synergistic effect between different components when compared with literature data for individual constituents.In Acute Toxicity of Behavaorial study were done 14 days following the CSP extract treatment, no deaths or signs of toxicity were observed in the experimental animals. According to the relative health study the oral administration of CSP is not toxic in a single dose.Biochemical and Hematological Analyses: The hematopoietic system is a critical target for toxic agents, and thus is an important indicator of the physiological and pathological status of humans and animals, the results demonstrated the practical absence of CSP extract toxicity in vitro and in vivo, drawing more attention to its antileukemic activity and the need for further investigation into its mode of action and limitations. Molecular Docking: Ligand Preparation, All chemical structures were retrieved as SDF 3D files from PubChem database (catechin CID: 9064; epicatechin CID: 72276; epicatechin gallate CID:107905; epigallocatechin CID:72277; gallocatechin CID:65084; oleuropein CID:5281544; rutin CID:5280805; chlorogenic acid: 1794427; vanillic acid CID:8486; nilotinib CID: 644241; danusetib CID: 11442891; quizartinib CID: 24889392) and then converted into a PDBQT format with AutoDockTools v1.5.6 [60] for the docking simulation. Preparation of Receptors: The receptors were prepared by deleting water molecules, the default ligand, and heteroatoms using the Discovery Studio Visualizer v21 for windows [62]. AutoDockTools was used to open the updated receptors and apply polar hydrogen atoms and Gasteiger charges before converting them to the PDBQT format for further docking simulations. Docking Simulations: AutoDockTools was used to specify the grid box size for each receptor, and AutoDockVina [63] was used to run the docking simulations for different ligands and the four receptors, Discovery Studio Visualizer v21 was used to generate images of the protein–ligand complexes. Statistical Analysis: Statistical analysis was carried out using Student's t-test and ANOVA. A probability value of less than 0.05 was chosen as the criterion of statistical significance.

Conclusion: This study demonstrate that the effects of the polyphenolic extract of C. sativum seeds on two leukemic cell lines (K562 and HL-60). It also demonstrated a dose-dependent cytotoxicity against the tumor cells and no effect on the viability and growth of a normal cell line (Vero). No signs of toxicity during the in vivo acute toxicity study demonstrated that CSP ais a very safe and effective combination of biologically active molecules. All CSP components interacted positively with the ABL kinase, ABL1, BCL2, and FLT3 receptors in the tumor cells, Taking into a consideration all the data obtained in the in vitro and in vivo experiments together with in silico results, the antileukemic activity of the CSP extract could be mainly attributed to a synergic combination of the catechins and rutin. catechins directly associate with cellular targets, that is the cell surface receptors.

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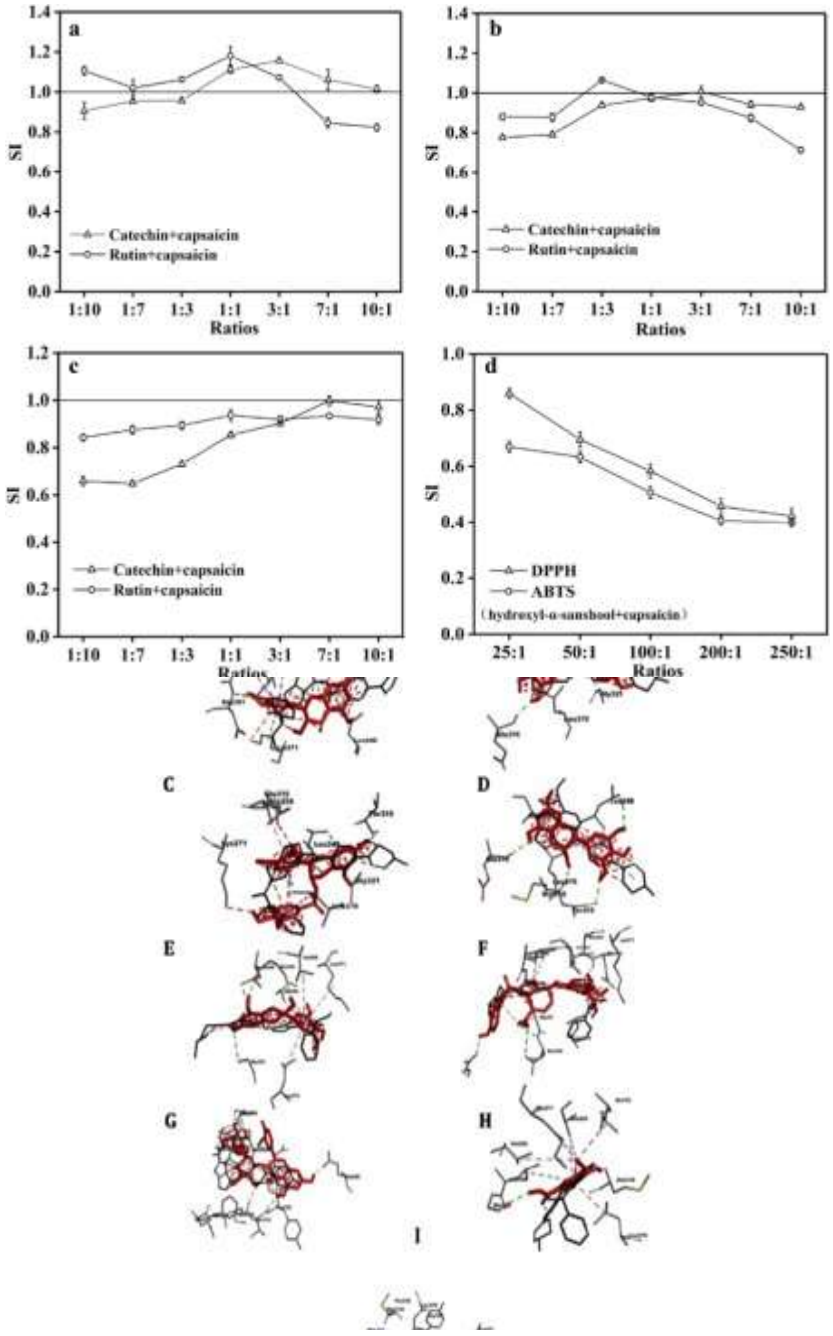

IV. CONCLUSION:

Focusing on green leafy vegetables and their potential anti-cancer properties, based on in-silico studies. Green leafy vegetables have been extensively studied for their potential health benefits, particularly in cancer prevention and treatment. In recent years, in-silico methods such as docking, homology modeling, molecular dynamics, and 3D QSAR have emerged as valuable tools to investigate the bioactive compounds present in these vegetables and their interactions with cancer-related molecular targets.

1.Effectiveness of Green Leafy Vegetables: Green leafy vegetables are rich sources of phytochemicals, vitamins, and minerals known for their antioxidant and anti-inflammatory properties, which are beneficial in combating cancer.2.Global Significance of Cancer: Cancer remains a

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significant global health challenge, necessitating the development of effective and safe treatments.3.Advantages of In-silico Studies: In-silico studies allow for a comprehensive exploration of the molecular mechanisms underlying the anti-cancer activity of compounds found in green leafy vegetables, offering insights into potential therapeutic benefits.4.Key Findings from Docking Studies: Through docking studies, specific bioactive compounds such as [mention specific compounds if applicable] have shown promising interactions with cancer-related proteins and pathways, suggesting their potential as anti-cancer agents.5.Future Directions: Further in-silico studies and subsequent experimental validations are essential to confirm the efficacy and safety of these compounds as cancer therapeutics. Integration of computational and experimental approaches will facilitate the development of novel treatments derived from green leafy vegetables.In conclusion, in-silico studies provide valuable insights into the anti-cancer potential of green leafy vegetables, highlighting their role as a promising source of natural compounds for future cancer therapies. Continued research efforts in this area hold promise for advancing our understanding and treatment options in oncology.This conclusion summarizes the current state of research on green leafy vegetables and their potential anti-cancer effects, emphasizing the role of computational methods in exploring their therapeutic applications. Adjustments can be made based on specific vegetables, compounds, or findings discussed in your study.

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