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Computational investigation of the anticancer potential of *Sorghum bicolor* and *Setaria italica* phytochemicals against dihydrofolate reductase (DHFR) enzyme

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Abstract

Breast and prostate cancer holds the position of foremost contributors to mortality. Dietary therapies for accompanied by medication are widely recognized as a potential method to successfully tackle cancer. Millet grains are the most ancient food, a perfect combination of proteins, carbohydrates, fiber, macronutrients, micronutrients, and vitamins. This study aims to examine the anticancer potential of *Sorghum bicolor* (Sorghum) and *Setaria italica* (Foxtail) phytochemicals. The 50 phytochemicals of sorghum and foxtail millets were retrieved through a literature survey and docked to the Dihydrofolate reductase (DHFR), an enzyme essential for cell growth and proliferation. The top-scoring phytochemicals were filtered and further investigated with active-site residue interaction, drug-likeness, and pharmacokinetics analysis. The ligand stability with the DHFR was evaluated through density functional theory (DFT) based HOMO and LUMO calculations. The results show that caffeic acid, ferulic acid, hesperetic acid, stigmasterol, Cis-p-Coumaric acid, and luteolinidin attained greater stability within the active site of DHFR. These phytochemicals showed a docking score of − 6.4 kcal/mol, − 6.4 kcal/mol, − 6.1 kcal/mol, − 6.4 kcal/mol, − 5.4 kcal/mol, and − 6.7 kcal/mol with DHFR (PDB ID:1BOZ) and flutamide and capecitabine have docking score of − 7.5 and − 8.1 for 1BOZ and − 7.4 and − 7.1 with DHFR (PDB ID:1OHK) respectively. The dynamic interaction at the molecular level validated the stability of these phytochemicals against both DHFR target proteins along with excellent drug-likeness and pharmacokinetic properties. However, the current findings were proven and validated through in-silico experiments to validate above identified phytochemicals as DHFR inhibitors, so millets are used as therapeutics for breast and prostate cancer.

Keywords *Sorghum bicolor* (Sorghum), *Setaria italica* (Foxtail), Breast cancer, Prostate cancer, Dihydrofolate reductase, Molecular docking , Density functional theory , Drug-likeness

Introduction

The food security is among the looming challenges for the twenty-first century which is flattering precariously through population growth and climate change. The United Nations forecasts a nine billion population growth by 2050 globally. The continents are witnessing a day by day increment in hunger, drought, and climate change conditions jeopardizing food security. The Norman Borlaug-led miracle seed green revolution

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nowadays needs another agricultural revolution. The Millets contribute as a reliable food source option for combating global hunger in the context of rapidly shifting climate conditions. The Food and Agricultural Organization of the United Nations defined Millet is as a cumulative term denoting to a number of small-seeded annual grasses cultivated as grain crops predominantly on marginal dry lands of temperate, tropical, and subtropical regions. Millets come into view as climate resilient and superior photosynthetic efficiencies crops over staple ones (Mehta et al. 2024).

During its 75th session in March 2021, the United Nations General Assembly officially designated the year 2023 as the International Year of Millets (IYM 2023) to shed light on millets nutritional and ecological importance. The extended purpose of IYM2023 is to draw policymakers, researcher and layman attention to millets potential for food security. Millets represent a viable source for nations seeking to enhance their self-sufficiency and diminish their dependence on imported cereal grains (Poshadri et al. 2023). Millets, staple crops in semiarid tropics, are thriving due to their high nutrient content and tolerance to extreme weather conditions. With concerns about lifestyle diseases arising due to refined diets, modern consumers are increasingly choosing millet as an alternative to wheat and rice, especially during COVID-19.

Millets, poaceae family members are ancient staple sustainable cereal crops foremost domesticated and cultivated by prehistoric humans Millets and other cereal grains are major food source for economically challenged parts of Asia and Africa, accounting for 75% of total calorie consumption. These regions produce 14.2 and 12.4 million tonnes of millet on average per year (Belton and Taylor 2004). India is the leading worldwide millet producer, accounting for almost 80% of overall millet output (Farsund et al. 2015).

Based on economic significance, cultivation, and consumption share, millets are grouped into two categories minor millets and major millets. The Pearl Millet (Bajra) (*Pennisetum glaucum (L.)*, Little Millet (Sorghum) (*Panicum sumatrense L*), Amarnath (Proso Millet) (*Amaranthus cruentus*), Kodo Millet (*Paspalums crobiculatum L.*), Finger Millet (Ragi) (*Eleusine coracana L*), Barnyard Millet (Sawan) (*Echinochola frumentacea L*), Foxtail Millet (Kakun) (*Setaria italic L*), Teff Millet (Abyssinian lovegrass) (*Eragrostis tef*), and Browntop Millet (*Panicum ramose L*) are imperative millets cultivated globally. Millets are disseminated as Nutri cereals or superfoods due to their richness in proteins, dietary fibers, antioxidants, vitamins bioactive phytochemicals, polyphenols and minerals. The genomic diversity of millets helps to thrive in diverse agricultural domains (Maharajan et al. 2024).

Sorghum (*Sorghum bicolor*) is a staple energy source for resource-limited inhabitants of Globe. It is a genotypic and phenotypic diverse, climate-resilient, drought-tolerant and multipurpose (food, fiber, fuel, and feed) crop (Bakari et al. 2023) (Okoye et al. 2014).

Foxtail millet (*Seteria italica*) is the oldest cultivated crop whose bio-functional phytochemicals show antihyperglycemic, anticancerous, antioxidant, anti-inflammatory, antihyperlipidemic medical prospects properties along with substantial proportions of carbohydrate, protein, fibers, and minerals (Kalsi and Bhasin, 2023).

Cancer is a cumulative disease term in which cells start uncontrolled cell division followed by invasion and spread to other cells and tissues. Sometimes disruptions in gene expression can cause erratic cellular proliferation, leading to the abnormal increase of cells. Cancer has been an ongoing public health concern throughout history, appearing as a top cause of death in advanced and developing countries worldwide. Current data analytics indicate a projected increase, with an estimated 23.6 million additional cancer cases per year by 2030 (Bray et al. 2018).

The phytochemicals are biologically active nutritive of plants. The alkaloids, phenolic, nitrogen-containing, organosulfur, and carotenoids are major groups of bioactive phytochemicals. The phytochemicals are potential agents to combat diabetes and cardiovascular diseases The FMBP, class III peroxidase protein extracted from foxtail millet was shown anti-colon cancer properties in-vitro and in-vivo experiments on mice. The FMBP arrests cancerous colon epithelial cells in the G1 phase whereas normal epithelial cells remain unaffected. The FMBP has shown effects on epithelial mesenchymal transition by down regulation of JAK1 signalling pathway followed by accumulation of more ROS in cancerous colon epithelial cells (Shan et al. 2014).

Another similar research proved that flavonoids, carotenoids and phenolics extract of foxtail millet arrest breast cancer cell lines in G2/M phase along with DNA fragmentation. Thus extracts show antiproliferative potential. The Kodo and pearl millet bran extracts showed anti-cancerous property in HT-29 cancer cell lines. The research study concluded that phytochemicals extracted from Proso and barnyard millets induce apoptosis and cell proliferation in the HT-29 human colon cancer cell line and arrest cancerous cells in Go/G1 phase (Ramadoss and Sivalingam, 2020) (Gupta et al. 2023).

Phytate phytochemical extracted from millets shows antiproliferative activity and downregulates NF-kB, P13K/MAPK signaling pathways in breast cancer cell lines (Shukla et al. 2023) Several systematic and review articles concluded that millets, Wonder grains encompass nutritional, health-promoting, anti-inflammatory and anticancerous properties. Millets are accessible

functional, low-cost foods that gaining the attention of industry to common people (Samtiya et al. 2022) (Nani et al. 2015).

Growth factor receptors (GFRs) are proteins that play important roles in many aspects of cell behavior, including tumor development, metastasis, angiogenesis, cell survival, apoptosis, cell motility, cellular differentiation, organ creation, neovascularization, and chemotherapy resistance(Tiash and Chowdhury 2015). These receptors are activated when they bind to certain ligands, commonly known as growth factors (Ornitz and Itoh 2015).

DHFR is a ubiquitous enzyme found in all organisms that catalyzes the conversion of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF). This process requires a precise hydride transfer from the NADPH cofactor to the pterin ring's C6 atom, followed by protonation at N5. DHFR plays a vital role in controlling cellular reservoirs of THF and its derivatives, which are required for the synthesis of purines and thymidylate, which are required for cell proliferation and growth(Schnell et al. 2004). Notably, this enzyme has received a lot of attention as a potential target for anticancer medicines and antibiotics(Chen et al. 2009). Anticancer medication development includes strategically disrupting certain proteins or pathways that are intricately engaged in cancer growth. As stated in (Siegel et al. 2023), breast cancer is one of the main causes of cancer-related death in women, while prostate cancer is similarly prevalent in men. Research has shown that DHFR is overexpressed in certain kinds of breast cancer. Furthermore, studies have revealed that inhibiting DHFR in prostate cancer cells may cause cell cycle arrest and death, thus slowing tumour development. Visual presentation of our work is shown below in Fig. 1

Materials and methods

Identification of the Plant's compounds, and their preparation

A thorough review of the available literature resulted in the identification of around fifty unique plant millet chemicals within *Sorghum bicolor* (Sorghum) (Kauther et al. 2020) (de Morais Cardoso et al. 2017) (Shih et al. 2007) and *Setaria italica* (Foxtail) (Sharma et al. 2021) (Zhang et al. 2021) (Nivedita et al. 2022). The 2D/3D structures of these identified phytocompounds were obtained in SDF format from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) chemical database, resulting in a complete library of chemical compounds as depicted in.

Figure 2Furthermore, two established medications, flutamide linked to prostate cancer and capecitabine to breast cancer, were obtained from the PubChem database with unique identities PubChem ID CID_3397 and CID_60953, respectively, for comparative comparison with the phytochemicals. These medications were also obtained in 3D SDF format. The ligands were converted into the PDB format using the PyRx Software (https://pyrx.sourceforge.io/downloads), making them suitable for further docking studies. This was followed by the construction of torsion requirements required for correct binding, which was accomplished through the use of AutoDock 4.2.6 parameters.

Source of protein crystal structures and their preparation

The protein X-Ray structures of DHFR a ubiquitous enzyme, were obtained from the protein data bank repository (http://www.rcsg.org). The unique identification numbers are PDB ID:1BOZ (Gangjee et al. 1998) and PDB ID: 1OHK(Cody et al. 1997).An array of preliminary processes were performed on the protein structures using

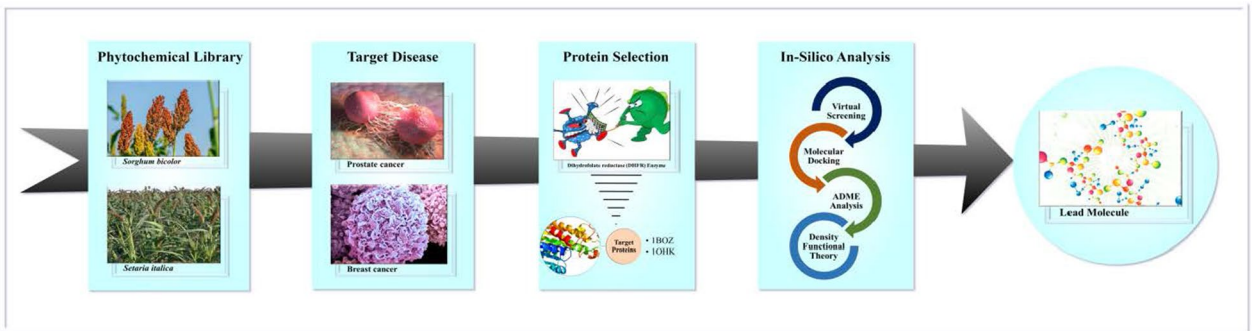


Fig. 1 Graphical abstract of research

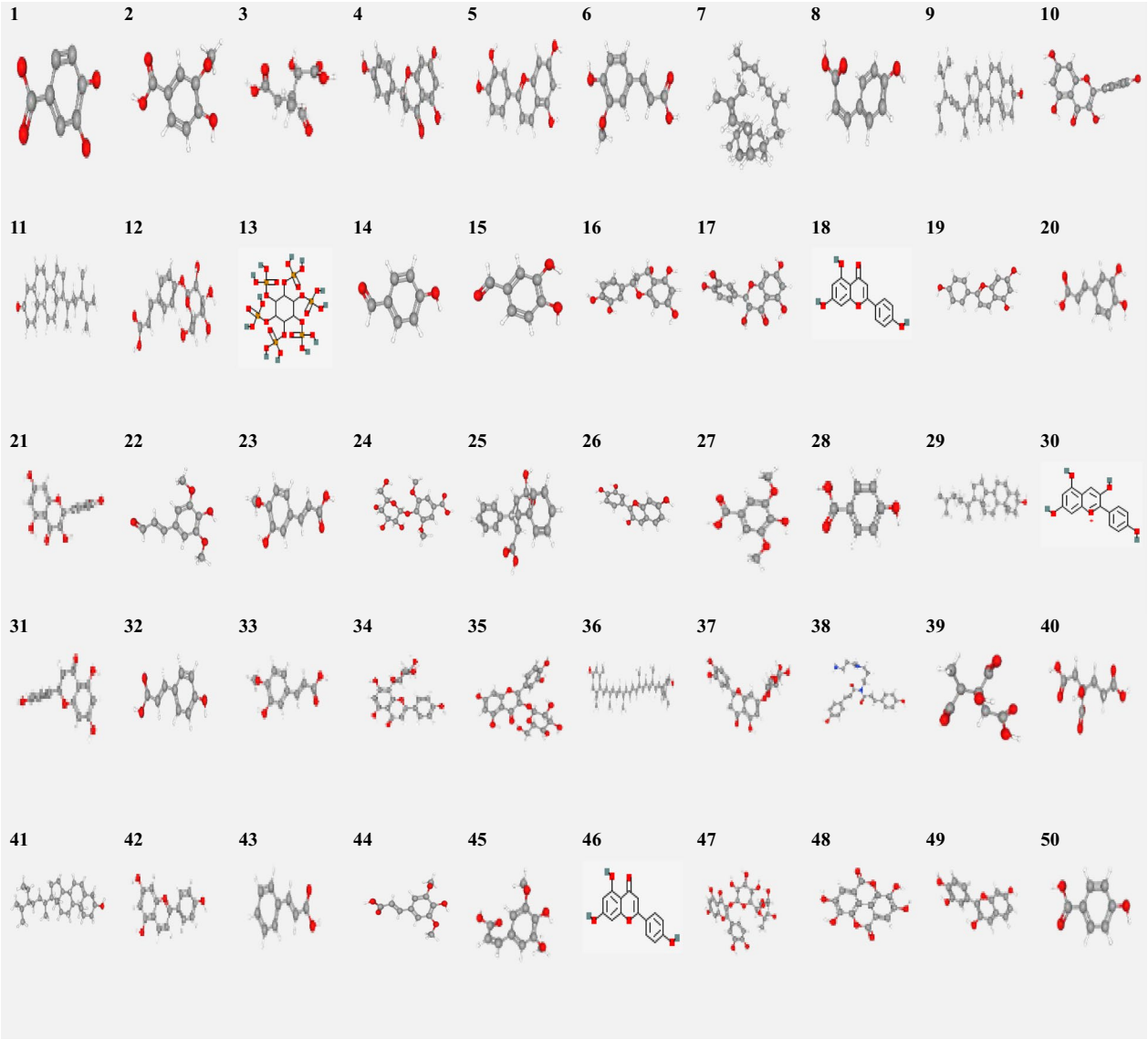


Fig. 2 Figure 3D Structures of 50 phytochemicals used in this study. (1) 3,4-Dihydroxybenzoic acid, (2) Vanillic Acid, (3) Truxillic acid, (4) Naringenin, (5) Luteolinidin, (6) Ferulic acid, (7) Squalene,(8) cis-p-Coumaric acid, (9)Stigmasterol, (10) Kaempferol,(11) Ergostanol,(12) trans-p-Coumaric acid (13) 4-glucoside,Phytic acid,(14) 4-Hydroxybenzaldehyde,(15) Protocatechualdehyde,(16) Luteoforol,(17) Taxifolin,(18) Peonidin,(19) Apigeninidin,(20) CaffeicAcid,(21) Quercetin,(22) Sinapaldehyde,(23) Zeaxanthin,(24) Glucosyringic acid,(25) 2-Methylisocitric acid,(26) Fisetinidin,(27) 4-Hydroxybenzoic acid, (28) Syringic acid, (29) Campesterol, (30) Pelargonidin, (31) Apiforol,(32) p-Coumaric acid, (33) Isoferulic acid, (34) Vitexin, (35) Isoquercetin, (36) Lutein,(37) (2S)-eriodictoyl-7-O-beta-D-glucopyranosiduronic acid, (38) Di-p-coumaroylspermidine, (39) 2-Methylcitric acid, (40) Homocitric acid, (41) Beta-Sitosterol,(42) 2-(4-Hydroxyphenyl)chromenylium-5,7-diol, (43) Cinnamic acid,(44) Sinapinic acid,(45) cis-Sinapic acid, (46) Apigenin,(47) Rutin,(48) Ellagic Acid, (49) 3,3′,4′,7-Tetrahydroxyflavylium, (50) N-caffeoyl-N′-feruloyl spermidine

AutoDock 4.2.6. This included removing water molecules and adding polar hydrogen atoms, as well as Kollman's charges. Following that, the changed protein structures were stored in the PDBQT format to ensure compatibility with further docking investigations.

Molecular docking studies and virtual screening

Following the careful preparation of proteins and ligands, a molecular docking method was carried out using Autodock 4.2.6.. Specific dimensions were defined along the grid box of 1OHK was used with center_x = 1.079,

centre_y = − 12.26, centre_z = 37.468 and 1BOZ with center_x = 24.397, centre_y = 12.818, centre_z = − 1.563, and the size_x = 20, size_y = 22, size_z = 20 for both receptor proteins respectively, while keeping a resolution of 0.500, to promote successful binding interactions.. The grid layout was precisely centered to maximize the

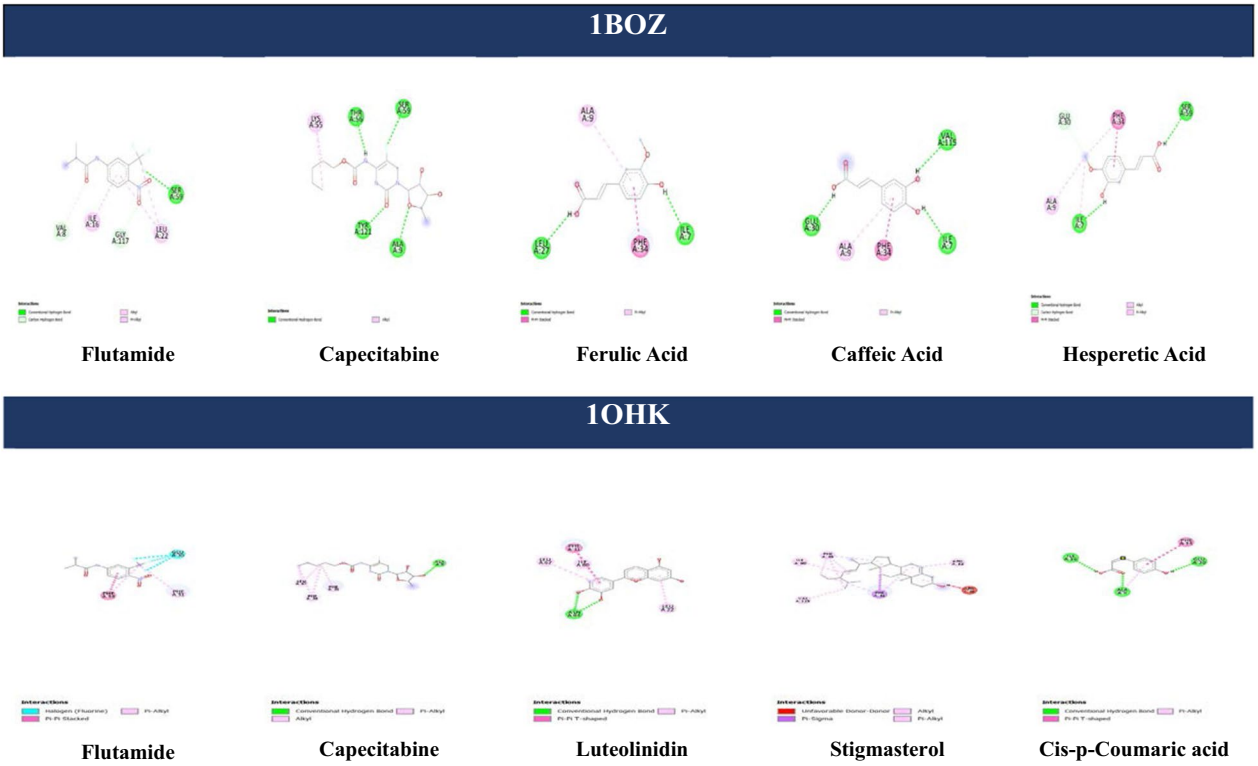


Fig. 3 2D interaction of the selected phytochemicals with 1BOZ and 1OHK

Table 1 Binding parameters between ligands and target protein 1BOZ

Phytochemicals with their pubchem ID	Source	Binding energy (Kcal/mol)	Inhibition constant (μm)	Amino acids involved in hydrogen bonding
Caffeic acid 689043	*Sorghum bicolor*	− 6.4	0.002	Ile7, Ala9, Glu30, Phe34, Val115
Ferulic acid 445858	*Setaria italica*	− 6.4	1.2	Ile7, Ala9, Leu27, Phe34
Hesperetic acid 736186	*Setaria italica*	− 6.1	10	Ile7, Ala9, Glu30, Phe34, Ser59

Table 2 Binding parameters between ligands and target protein 1OHK

Phytochemicals	Source	Binding energy (Kcal/mol)	Inhibition constant (μm)	Amino acids involved in hydrogen bonding
Stigmasterol 5280794	*Sorghum bicolor*	− 6.4	0.0891	Phe31, Phe34, Ile60, Val115
Cis-p-coumaric acid 1549106	*Setaria italica*	− 5.4	100	Ala9, Ile16, Glu30, Phe34
Luteolinidin 441701	*Sorghum bicolor*	− 6.7	1.275834	Leu22, Phe31, Ile60, Asn64, Leu67

creation of favourable docking conformations (Mendie and Hemalatha 2022). This grid setup was then saved as a (.gpf) file and executed by Autogrid.

Following that, docking calculations were started using the Lamarckian genetic method, with a standard of ten separate runs. The docking computations that resulted were saved in a (.dpf) file. Following the conclusion of the Autodock computations, the definitive docked results were obtained and stored in a (.dlg) file format. Tables 1 and 2 provides useful information, such as binding residues, binding energy, and inhibition constants. The structures representing the interactions between ligands and proteins were visualized using Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download) to visually examine these interactions.

In silico ADME analysis

The chemical compound's drug-likeness, physicochemical qualities, and pharmacokinetic properties were comprehensively evaluated. This assessment included key pharmacokinetic characteristics such as absorption, distribution, metabolism, and excretion, which were examined in the ligands using the SwissADME platform (http://www.swissadme.ch/index.php). This detailed study provides significant insights that may be used to guide and improve the medication research and development process.

Single-point calculation to determine the reactivity and inertness of compounds

The fundamental ideas of density functional theory (DFT) are derived from the Hohenberg–Kohn theorem, which states that the energy of a system may be described as a function that is entirely dependent on the electron density. To investigate this, chosen chemical components derived from *Sorghum bicolor* (Sorghum) and *Setaria italica* (Foxtail) were meticulously geometrically optimized in Maestro using the Jaguar fast engine. The functional set used in this optimization procedure was Becke's three-parameter exchange–correlation functional hybrid (B3LYP), which was supplemented with a 6-31G basis set, resulting in DFT as the preferred level of theory.

Following this optimization, the investigation focused on the geometrically improved compounds surface characteristics. This involved computing molecular orbitals in the gaseous phase, which provided important insights into the electrical structure and behavior of the compounds.

Results

Docking and virtual screening analysis

That growth factor receptors play in the start and progression of cancer, it is critical to develop therapeutic candidates that can effectively inhibit growth factors at receptor sites while causing minimum or insignificant effects elsewhere. The intensity of the interaction between ligands and receptors is governed by binding affinity, which is measured by binding energy. Notably, lower binding energy indicates a stronger binding affinity (McConkey et al. 2002).

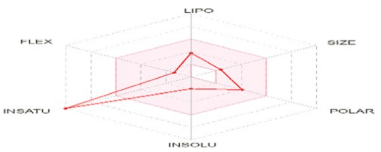
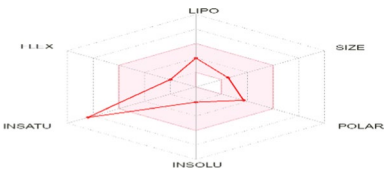
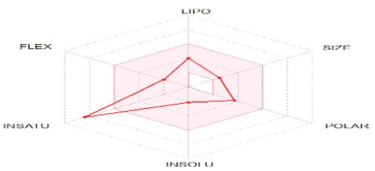
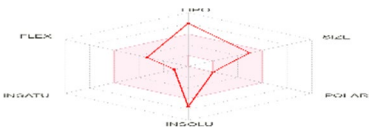
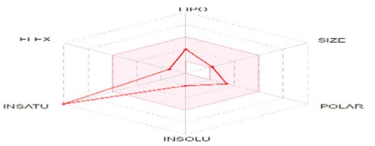
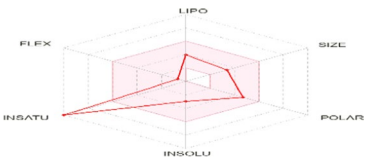
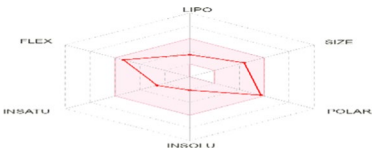
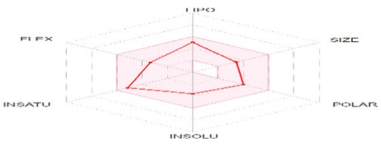
To achieve this goal, 50 bioactive chemicals were subjected to docking interactions with the DHFR protein in a thorough virtual screening approach. The Flutamide (for Prostate cancer) and Capecitabine (for breast cancer) is docked as positive control to the DHFR receptor protein. The phytochemicals were sorted out on the basis of binding energy. The post-docking investigation were performed for binding energy (in kcal/mol), the number of hydrogen bonds, the inhibitory constant (expressed in M/nM), and the amino acids intricately involved in hydrogen bonding interactions between phytochemical ligands and target protein.

At the end of the molecular docking analysis this study identifies the following compounds with high binding affinity with DHFR; caffeic Acid, ferulic Acid, hesperetic Acid, stigmasterol, cis-p-coumaric acid, and luteolinidin. These compounds showed a docking score of − 6.4 kcal/mol, − 6.4 kcal/mol, − 6.1 kcal/mol, − 6.4 kcal/mol, − 5.4 kcal/mol, and − 6.7 kcal/mol with Dihydrofolate reductase and flutamide and capecitabine have docking score of − 7.5 and − 8.1 for 1BOZ and − 7.4 and − 7.1 for 1OHK respectively.

Pharmacokinetic and drug likeness screening of phytochemical

The path to drug discovery is inherently difficult. The financial investment necessary for drug research is significant, and the potential of developing medicinal molecules with low toxicity is particularly difficult. It is worth noting that only a small percentage of prospective lead compounds get approved by the FDA (Wishart 2007). A drug lead can be terminated at any point of the development process owing to a variety of issues such as inefficacy, unwanted effects, toxicity concerns, poor absorption, or inefficient clearance. Unfortunately, it has been discovered that the more promising a medication lead seems, the more expensive it is to cease its research. Prediction or modelling of ADME (absorption, distribution, metabolism, and excretion) is one potential route in this complex process. ADME data provides useful information on how medicine will interact with the body. While a pharmacologically promising lead may demonstrate great performance in vitro, unfavourable ADME data typically result in the lead's termination. Robust ADME prediction, in essence, serves as a vital

Table 3 Lipinski's rule of five and radar chart of the selected phytochemicals

Properties	MW(g/mol)	Rotatable Bonds	HBD	HBA	Log Po/w (Cons)	Molar refractivity	TPSA (Å^2)	RADAR Chart
1BOZ								
689043 Caffeic acid	180.16	2	3	4	0.97	47.16	77.76 Å^2	
445858 Ferulic acid	194.18	3	2	4	1.62	51.63	66.76 Å^2	
736186 Hesperetic Acid	194.18	3	2	4	1.79	51.63	66.76 Å^2	
1OHK								
5280794 Stigmasterol	412.69	1	1	5	5.08	132.75	20.23 Å^2	
1549106 Cis-p-Coumaric acid	162.16	2	2	3	1.11	45.13	57.53 Å^2	
441701 Luteolinidin	274.24	1	4	5	−1.95	74.15	94.06 Å^2	
Standard drugs								
60953 Capecitabine	359.35	8	3	8	2.24	85.25	122.91Å^2	
3397 Flutamide	276.21	5	1	6	1.85	64.19	74.92 Å^2	

filter, impacting the development route of possible drug candidates.

The results reported in Table 3 offer a complete summary of the chemical compounds drug-likeness and pharmacokinetic properties. The examination of physicochemical parameters, which include important chemical and physical factors such as molecular weight, hydrogen bond donors, hydrogen bond acceptors, and octanol/water partition coefficient (QPlogPo/w), helps to determine the drug-likeness of the compounds. The

calculated molecular weights of the plant compounds vary from 164.20 to 412, with hydrogen bond donors and acceptors ranging from 1 to 4 and 3 to 8, respectively. Furthermore, the octanol/water partition coefficients range between 0.79 and 5.08, which are all within acceptable limits.

Significantly, all of these compounds fulfil more than two of Lipinski's rule of 5 (RO5) qualifying requirements (Lipinski et al. 2001), as they have a maximum of 5 hydrogen bond donors, a maximum of 5 hydrogen bond acceptors, a molecular weight less than 500 kDa, and log P values less than 5. As a result, all the crystallized ligand that binds to the proteins exhibit drug-like properties.

This assumption is supported further by Human Oral Availability (HOA) testing, which demonstrates that these drugs have medium to high availability. With the exception of Stigmasterol, all ligands had high GIA values. It is worth noting that crucial therapeutic drugs usually operate as p-glycoprotein substrates, which are frequently linked with decreased drug absorption, permeability, oral bioavailability, and total drug retention. P-glycoproteins are highly expressed in cancer cells, posing a substantial challenge to cancer therapy. This overexpression causes drug efflux, which significantly reduces the efficiency of treatment (Bansal et al. 2009). As a result, in the context of cancer treatment, ligands that do not operate as p-glycoprotein substrates stand out as the most attractive possibilities.

The majority of phytochemicals, as shown in Table 4, have non-inhibitory effects on CYP3A4 and CYP1A2, which are key members of the cytochrome P450 family of drug-metabolizing enzymes. Cytochrome P450 enzymes are essential in the metabolism of several medications. Their interactions with pharmaceuticals can result in two distinct outcomes: rapid metabolism if the drugs function as substrates for certain CYP enzymes, resulting in drug induction; or drug accumulation if the drugs act as inhibitors of CYP enzymes, resulting in inhibition. Both of these possibilities are undesired (Ji et al. 2020). As a result, using in silico research to identify possible interactions between chemicals or medications and certain CYP isoenzymes is critical during the drug development process. The substances values for these parameters are impressively close to established standards, indicating their outstanding pharmacokinetic properties. This validates their ability to efficiently transfer metabolites to specific locations, indicating a distinct pharmacological significance.

The Bioavailability Radar determines a compound's drug-likeness in real-time. The pink region in figure as depicted in Table 3 represents the best parameter range. A phytocompound that falls inside this pink region on the radar map is considered drug-like. This estimates the potential for oral bioavailability of the ligands. Flexibility (FLEX) and polarity (polar) are important factors influencing chemical bioavailability. Rotatable bonds impact flexibility, with substances with more than ten such bonds having limited oral bioavailability. Polarity, as assessed by topological polar surface area (TPSA), reveals that drugs with TPSA between 20Å^2 and 130 Å^2

Table 4 In silico pharmacokinetics of ligands using Swiss ADME

Properties	Log S(ESOL)	GI absorption	BBB permeant	P-gp substrate	CYP1A2	CYP3A4	CYP2C19	Bioavailability score
1BOZ								
Caffeic acid 689043	Very Soluble	High	No	No	No	No	No	0.56
Ferulic acid 445858	Soluble	High	Yes	No	No	No	No	0.85
Hesperetic Acid 736186	Soluble	High	Yes	No	No	No	No	0.85
1OHK								
Stigmasterol 5280794	Poorly Soluble	Low	No	No	No	No	No	0.55
Cis-p-Coumaric acid1549106	Soluble	High	Yes	No	No	No	No	0.85
Luteolinidin 441701	Soluble	High	No	Yes	Yes	No	No	0.55
Standard drugs								
Capecitabine 60953	Soluble	High	No	Yes	No	No	No	0.55
Flutamide 3397	Soluble	High	Yes	No	Yes	No	Yes	0.55

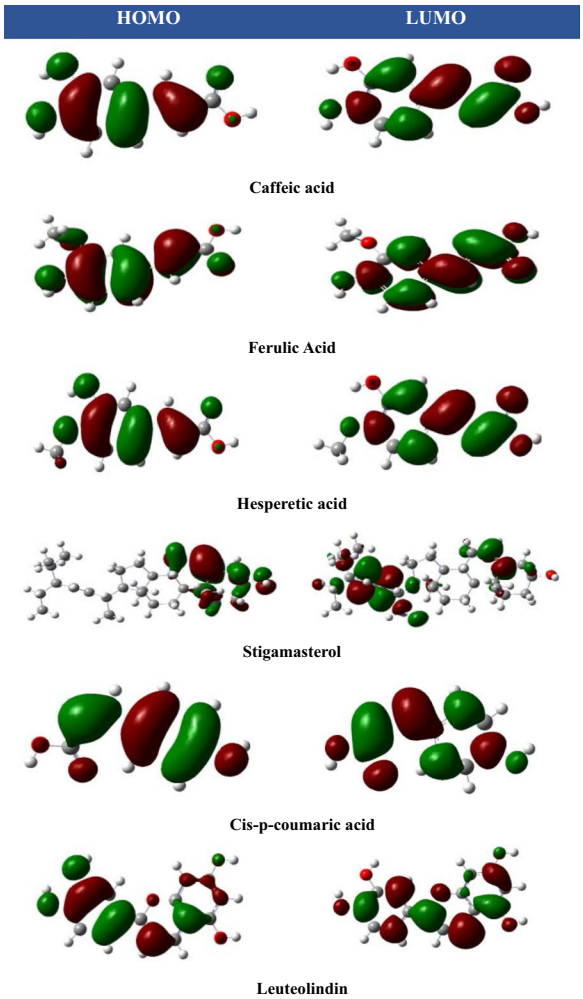


Fig. 4 The HOMO and LUMO diagrams of (1) Caffeic acid, (2) Ferulic Acid, (3) Hesperetic acid, (4) Stigamasterol, (5) Cis-p-coumaric acid, (6) Leuteolindin

have a high oral bioavailability (Khan et al. 2017).Therefore majority of phytochemicals meet radar plot requirements, showing their potential for oral bioavailability.

Homo lumo calculation

The frontier molecular orbital (FMO), which derives from the molecular orbitals with the greatest occupied (HOMO) and lowest unoccupied (LUMO) energies, plays an important role in showing compounds active sites during molecular interactions. This smart visualization assists in understanding the dynamic interaction of molecules. Figure 4 show the HOMO and LUMO areas for the compounds from the nine plants. LUMO refers to orbitals with the energy capacity to receive electrons, whereas HOMO refers to the area inside compounds with the greatest energy and most reactive electrons. As a result, it has been proposed that these bonding orbitals form strong interactions between ligands and receptors at these precise sites (Iwaloye et al. 2022).

Table 5 shows the HOMO/LUMO values predicted by DFT for the substances. The HOMO orbitals of the compounds have energy levels ranging from − 0.26751 to − 0.33045 eV, whereas the LUMO orbitals have energy levels ranging from 0.06266 to 0.18089 eV.

As a consequence, Caffeic acid was found to have the lowest LUMO energy level at − 0.06266, while Stigamasterol had the highest LUMO energy level at 0.18089. To acquire a better understanding, the Frontier Molecular Orbital (FMO) band gap (measured in eV) was calculated by subtracting the LUMO value from the HOMO value. This calculation is particularly important since it identifies both highly chemically reactive and moderately inert substances. It is generally understood that decreasing the energy gap increases a molecule's chemical reactivity, whereas increasing the energy gap reduces reactivity (Olawale et al. 2022). This guiding concept was applied to the energy gap values calculated from the chemicals, resulting in Hesperetic acid being identified as the most chemically reactive molecule in the gaseous phase Table 5 Stigamasterol and Ferulic acid, on the other hand, appeared as the least reactive molecules among the tested candidates. The energy gap displayed by these plant-derived chemicals has the potential to reveal new insights into their interactions with biomolecules such as proteins. This study gives information on the processes driving these interactions and their possible metabolic consequences.

Discussion

Millet are staple diet source for developing nations since decades. Based on the nutritional profile, anti-inflammatory, anti-oxidant, hypocholesterolemic, hypoglycemic

Table 5 Calculation of frontier molecular orbital

Parameters	Caffeic acid	Ferulic acid	Hesperetic acid	Stigamasterol	Cis-p-coumaric acid	Leuteolindin
HOMO	− 0.31231	− 0.31126	− 0.30636	− 0.33045	− 0.30851	− 0.26751
LUMO	0.06266	0.06438	0.06496	0.18089	0.06554	0.12469
Band gap	− 0.37497	− 0.37564	− 0.37132	− 0.51134	− 0.37405	− 0.3922

and anti-carcinogenic attributes of millet seed and derivative bioactive are considered miracle functional foods. Numerous research findings proved that millet seed derived phytochemicals slows down the metabolic disorder progression and some chronic diseases. In this article, *Sorghum bicolor* and *Seteria italica* phytochemicals anticancerous potential are discussed. The inhibitors of dihydrofolate reductase (DHFR), thymidylate synthase and serine hydroxyl methyltransferase of folate metabolic cycle are crucial targets for cancer treatment. Moreover, DHFR is involved in both DNA synthesis and amino acid metabolism, highlighting its importance as a therapeutic target in a variety of illnesses (Luo et al. 2006).

The folate metabolism inhibitors have received widespread attention for their effectiveness in treating cancer, rheumatoid arthritis, and microbial infections. This is primarily owing to their capacity to affect important enzymes such as thymidylate synthase, dihydrofolate reductase (DHFR), and serine hydroxymethyltransferase, all of which are linked to folate metabolism disorders. Notably, DHFR is involved in both DNA synthesis and amino acid metabolism, highlighting its importance as a therapeutic target in a variety of illnesses (Luo et al. 2006).

Rao et al. promising DHFR inhibitors were found utilizing in silico analysis. It was discovered that in human dihydrofolate reductase, Glu30 plays an important function in the active site by creating hydrogen bonds with inhibitors (Rao and Tapale, 2013). In our study we found similar hydrogen bond interaction in Hesperetic acid, Cis-p Coumaric acid obtained from *Setaria italica* which is also reported in (Zhang et al. 2023).The study found similar hydrogen bond interactions between Glu30 and both reference and newly discovered hit phytocompounds. It was discovered that the carboxylate oxygen of Glu30 establishes hydrogen bond interactions with the pyridopyrimidine ring found in inhibitor compounds (Rana et al. 2019).

The *Sorghum bicolor* and *Setaria italica* possess numerous bioactive phytochemical with diverse role from excellent nutrition to medicated properties. Keeping in view, the anticancerous activity of millet phytochemicals explored to cure cancer. To the best of our research investigation, this is first study reporting anticancerous activities via DHFR inhibition of hespertic acid, ferulic acid, stigmasterol, and caffeic acid.

Conclusion

In this study, molecular docking methods were used to identify six compounds isolated from *Sorghum bicolor* (Sorghum) and *Setaria italica* (Foxtail) for their inhibitory actions on Dihydrofolate reductase (DHFR) enzyme. Among these are Caffeic acid, Ferulic acid, Hesperetic acid, Stigmasterol, Cis-p-courmaric acid and Leuteolindin. The docking scores underwent a thorough post-docking examination to confirm the validity of these results. This study also used ADME predictions to show that these compounds had admirable drug-like qualities and pharmacokinetic characteristics. Hesperetic acid was found to be the chemical among the six that was found to be the most reactive when evaluated for reactivity in the gaseous phase. This finding emphasises the ability of bioactive substances to interact with growth factor receptors (GFRs) and block growth factors. In turn, this inhibition serves as a barrier to the growth of cancer cells against dihydrofolate reductase (DHFR) enzyme. This study implies that the lead compounds it has found have the potential for outstanding protein inhibitory activities, calling for more in vivo and in vitro research. These results also corroborate earlier in vivo studies on these phytochemicals, highlighting their promise as possible anti-cancerous options, as observed by other researchers in the area.

Author contributions
Kalpana Katiyar conceived and designed research. Anshika Gupta, Kalpana Katiyar and Akriti Verma performed all experimental analysis. All authors equally contributed in manuscript writing, editing and proof reading.

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Declarations

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