**PRECLINICAL STUDY**

# Analysing potent biomarkers along phytochemicals for breast cancer therapy: an in silico approach

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## Abstract

**Purpose** This research focused on the identification of herbal compounds as potential anti-cancer drugs, especially for breast cancer, that involved the recognition of Notch downstream targets NOTCH proteins (1–4) specifically expressed in breast tumours as biomarkers for prognosis, along with P53 tumour antigens, that were used as comparisons to check the sensitivity of the herbal bio-compounds.

**Methods** After investigating phytochemical candidates, we employed an approach for computer-aided drug design and analysis to find strong breast cancer inhibitors. The present study utilized *in silico* analyses and protein docking techniques to characterize and rank selected bio-compounds for their efficiency in oncogenic inhibition for use in precise carcinomic cell growth control.

**Results** Several of the identified phytocompounds found in herbs followed Lipinski's Rule of Five and could be further investigated as potential medicinal molecules. Based on the Vina score obtained after the docking process, the active compound Epigallocatechin gallate in green tea with NOTCH (1–4) and P53 proteins showed promising results for future drug repurposing. The stiffness and binding stability of green tea pharmacological complexes were further elucidated by the molecular dynamic simulations carried out for the highest scoring phytochemical ligand complex.

**Conclusion** The target-ligand complex of green tea active compound Epigallocatechin gallate with NOTCH (1–4) had the potential to become potent anti-breast cancer therapeutic candidates following further research involving wet-lab experiments**.**

**Keywords** Cancer cells · Herbal compounds · Phytochemicals · Docking · In silico analysis

## Introduction

Breast cancer as the most prevalent malignant type of cancer is the main driver of cancer-related deaths globally [1]. According to statistics, 2.3 million were diagnosed with breast cancer in 2020 globally, and the incidence rate of breast cancer between 2014 and 2018 has been gradually increasing by 0.5% annually each year [2, 3]. Surgical intervention is the primary form of treatment for breast cancer, whilst other treatments include chemotherapy, radiation, endocrine therapy, targeted therapy, and immunotherapy [4–6]. Notable alternatives are secondary metabolites from plants, which present accessible and effective natural substances with a range of bioactive constituents, including anti-cancer effects [7]. Numerous anti-cancer medications were discovered by years of intensive research into the underlying molecular mechanism of malignancies. Regrettably, most medications have substantial side effects, such as toxicity and therapeutic resistance [8–10]. By 2040, it is anticipated that there would be 16.4 million cancer-related deaths and 29.5 million new instances of cancer annually [11, 12]. Chemotherapy and radiotherapy are two examples of conventional cancer therapies with negative procedure consequences, such as functional impacts causing fatigue, nausea, anaemia, loss of oxygen, and emotional impact, such as stress [13–15].

Finding novel anti-cancer medications that are less toxic and have better therapeutic effects are now crucial. There has been a huge shift away from chemically derived pharmaceuticals and towards substances based on natural products, and many scientific works have begun to investigate the anti-cancer potential of phytochemicals [16]. By altering signalling

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pathways, the phytochemicals obtained from a variety of medicinal plants can stop tumour growth, aggressiveness, and motility of cancerous cells [17–23]. As a result, phytochemicals have received a lot of interest in recent years as potential agents for cancer therapy [24].

The basic Notch signalling system is essential for cell proliferation, division, and cell fate determination and is evolutionarily conserved across many organisms [25, 26]. Important processes like breast growth, healthy hematopoiesis, and colorectal epithelial maturation are affected by Notch [26–30]. Malignant growth is linked to the dysregulation of the Notch signalling cascade, with other epithelial malignancies and chronic intestinal inflammation also linked to the Notch signalling pathway [30–33]. Depending on the type of tumour, Notch signalling pathways have been observed to either enhance or hinder tumour growth through angiogenesis, cell cycle progression, differentiation, cellular metabolism, and immunological function [34–37]. According to a recent review, Notch receptors are intimately involved in the Notch signalling pathways oncogenic role in haematological malignancies, lung adenocarcinoma, breast cancer, and ovarian cancer [38]. Despite numerous publications linking Notch signalling to breast cancer, no clinical research on patients with breast cancer has been performed, with limited studies on the relationship between Notch-related gene expression and the clinical characteristics of breast cancer patients [39]. The improper functioning of the Notch signalling pathway has been associated with a range of ailments, such as cancer, cardiovascular disorders, and neurological conditions [40–42]. These diseases can be attributed to the disruption of normal cellular processes, resulting in abnormal growth, development, and function of various bodily systems [43]. As such, understanding the intricacies of Notch-based signalling is critical for the development of effective treatments and prevention strategies for these debilitating conditions. The Notch receptor consists of several domains, including the extracellular domain, transmembrane domain, and intracellular domain. The Notch receptor proteins (Notch 1, Notch 2, Notch 3, and Notch 4) all share a similar structure, consisting of various domains that contribute to their function [44, 45]. One of the key domains involved in ligand binding and activation is the extracellular domain, which contains multiple epidermal growth factor (EGF)-like repeats [46]. These repeats are responsible for interacting with the ligands of the Notch pathway and triggering downstream signalling events [47]. Phytochemicals can potentially interact with these EGF-like repeats, influencing the activation of the Notch receptor [48]. In cancer treatment, dysregulation of Notch signalling promotes survival and proliferation of cancerous cells, making inhibition of specific domains like the extracellular domain that binds to ligands beneficial for therapeutic purposes [49]. The extracellular domain is a critical component for ligand

recognition and receptor activation, leading to the release of Notch intracellular domain (NICD) and downstream cellular responses. Notch 1–4 receptors, expressed in various tissues, play distinct roles in development, homeostasis, and disease [50] with significant potential for therapeutic applications across various health conditions by inhibiting specific domains of the Notch receptor. However, research in this area is still work in progress, and any potential therapeutic interventions still require thorough testing and validation [51].

The Notch signalling pathway has gained significant attention in breast cancer therapy due to its multifaceted role in tumour development, progression, and treatment resistance [52, 53]. The four Notch receptors exhibit distinct functions in breast cancer, making them potential therapeutic targets. Notch 1 plays a crucial role in breast cancer by promoting cell proliferation and survival [54]. It is commonly overexpressed in various breast cancer subtypes, leading to chemotherapy resistance and stem cell preservation [55]. Targeting Notch 1 can hinder tumour growth, enhance chemotherapy efficacy, and reduce stem cell-like properties [56]. Notch 2's role in breast cancer is not as well understood as Notch 1, but has been known to impact cell fate decisions and differentiation in the mammary gland [57]. Notch 2 may regulate estrogen receptor signalling in hormone receptor-positive breast cancer and modulating its activity could impact hormone responsiveness and contribute to targeted therapies [58]. Notch 3 is often upregulated in HER2-positive and triple-negative breast cancers and is associated with aggressive phenotypes and poor outcomes [59]. Notch 3 signalling can enhance cell survival, invasion, and metastasis of cancerous cells. Suppressing Notch 3 has been investigated to limit metastasis and enhance cell response to chemotherapy [60, 61], as well as affect the tumour microenvironment and decrease activation of cancer-associated fibroblasts [62]. Notch 4's role in breast cancer is not well-defined compared to other Notch receptors, but has been linked to angiogenesis and vascularization, which may influence tumour growth and metastasis [63]. Expression of Notch 4 is associated with the basal-like subtype of breast cancer. Its involvement in promoting endothelial cell survival suggests a possible role in therapies that inhibit angiogenesis [64]. Notch 1–4 receptors have intricate and context-dependent roles in the development and progression of breast cancer. Targeting these receptors and the Notch signalling pathway shows promise in creating innovative therapeutic approaches in breast cancer therapy [51]. However, further research is necessary to understand the complex interactions and consequences of modulating Notch signalling in breast cancer cells.

Molecular categories for oncology biomarkers include different types, such as genetic, epigenetic, proteins, glycoproteins, receptors, and hormones [28, 31–33,

40]. The classification of tumour biomarkers includes pharmacodynamic, prognostic, diagnostic, and predictive types. Since their sensitivity and specificity offer excellent accuracy, protein biomarkers have been heavily relied upon in early diagnosis and treatment [65, 66]. Other classifications based on clinical characteristics are Type 0, Type I, and Type II biomarkers. Type 0 biomarkers linked to well-known clinical signs are used to track the diseases' natural histories. On the other hand, type I biomarkers are associated with the efficiency of pharmacological treatments. Secondary biomarkers, or type II biomarkers, were developed to take the place of clinical goals [67]. The estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are well-known biomarkers for predicting the subtype, prognosis, and treatment options for breast cancer patients [68–71, 78]. Recurrence rates and treatment effectiveness are assessed using biomarkers, such as CA15-3, HER2, CA 27.29, estrogen/progesterone receptors, urokinase plasminogen activator, and plasminogen activator inhibitor (PAI-1) [71, 79, 80].

The tumour protein Notch (1–4) have had limited research in breast cancer-related studies for drug discovery. Notch receptors when downregulated appear to have suppressive activity towards breast cancer [72–76]. As such, Notch receptor mutations are linked to poor prognoses and contribute to the pathophysiology of solid tumours and human haematological malignancies [52, 53]. A popular option for anti-cancer treatments is P53, which is mutated in most breast cancer cases [77]. Mutated P53 is another potential biomarker and therapeutic target for people with breast cancer, with a number of pharmaceuticals on the market that can restore the wild-type features of mutant P53 proteins with demonstrated anti-cancer efficacy [54, 55]. Whilst P53 was once thought to be "non-druggable," drugs that specifically target mutated P53 have recently appeared on the market [56, 57, 81]. A high level of complexity is involved in understanding the pharmacokinetics of protein biomarkers, with the development of suitable therapeutic treatments requiring substantial time and resources invested into the clinical decision-making [58, 59, 82, 83].

The initial stages of this study investigated various herbal constituents and their potential applications in the prevention of chronic ailments, with a focus on anti-cancer activity. Recent articles on the topic of herbs and spices that potentially exhibited anti-cancer and anti-tumorigenic properties were surveyed and studied. After careful collation of literature, a proposed investigation of selected phytochemicals was performed, with a specific focus on their potential to prevent the onset of breast cancer. Research has shown that Epigallocatechin gallate, an active compound present in Green Tea amongst other phytocompounds can induce apoptosis and inhibit the growth of several cancer types, such as colon, kidney, breast, brain cancers, and leukaemia

both in vivo and in vitro [84]. It has also shown that Epigallocatechin gallate has the ability to restrict the expansion of cancer cells, particularly breast cancer cells [85, 86]. Furthermore, a study on Asian-American women revealed that frequent consumption of green tea was linked to a lower risk of developing breast cancer [87]. Piperine from black pepper shown to inhibit the growth and motility of triple-negative breast cancer cells whilst leaving normal breast cells unaffected, but the clinical use of Piperine is limited by its hydrophobic nature and poor aqueous solubility [88, 89]. Ginger and its main bioactive compound, gingerol, have shown cytotoxic effects on various cancer types, including breast, cervical, colorectal, leukaemia, liver, lung, nasopharyngeal, ovarian, prostate, and retinoblastoma [90–92]. Black cumin's derivative Thymoquinone has been found to have positive effects on reducing carcinogenic effects in rats with mammary carcinoma when combined with melatonin and retinoic acid. Derivatives of black cumin have also been tested and shown to induce cell death by apoptosis in MCF-7/Topo breast carcinoma [93].

A comprehensive analysis performed on the inhibitory effects of phytochemicals on various stages of the signalling pathway indicated how these compounds impede the Notch pathway. Epigallocatechin gallate (Green tea) is a polyphenolic compound that can interfere with the Notch signalling pathway at multiple levels [94]. It is suggested that Epigallocatechin gallate may inhibit the ligand–receptor interaction by disrupting the binding of Notch ligands (such as Jagged or Delta-like) to the Notch receptor extracellular domain. Additionally, it might influence the γ-secretase complex, which is responsible for the second cleavage of the Notch receptor, thereby preventing the release of the NICD [95]. Moreover, it could affect the translocation of NICD to the nucleus of cancer cells and its interaction with transcriptional co-activators [96]. Piperine's inhibitory effects on the Notch pathway may involve interfering with the proteolytic cleavage of the Notch receptor. It could target the γ-secretase complex and inhibit the second cleavage step, preventing the release of NICD [88]. This action would subsequently hinder the formation of the Notch intracellular domain and its downstream transcriptional activities [97]. Gingerol's inhibitory mechanisms on the Notch pathway may encompass modulating the ligand–receptor interaction, preventing the ligand from binding to the Notch receptor [98]. Moreover, gingerol might disrupt the γ-secretase-mediated cleavage of Notch, impairing the generation of NICD. By doing so, gingerol could attenuate the downstream signalling events driven by NICD [99]. Thymoquinone's potential inhibition of the Notch signalling pathway might involve interfering with the ligand–receptor interaction, possibly by affecting ligand presentation or receptor binding [100]. Additionally, thymoquinone

could impact the γ-secretase-mediated cleavage of Notch, thus reducing the production of NICD [101]. This action would subsequently hinder the activation of downstream Notch target proteins.

Table 1 presents a candidate list of herbs with compounds containing unique anti-cancer or anti-tumorigenic properties. Amongst these herbs, no research to date has been done to identify the anti-cancer compounds found in Green tea, Black pepper, Black cumin, and Ginger against Notch inhibitors. Due to the limited literature available, the protein–ligand complexes that are investigated in this study have been involved in fewer research avenues. We used the CB-Dock2 server for docking and ranking the target-ligand complex based on their scoring function [102]. This study performed virtual screenings of various phytochemicals with the Notch protein to identify anti-tumour compounds from plants. ADMET was then used to perform drug-likeness predictions and molecular dynamic simulation analyses. Based on docking scores, the screened compounds were subjected to molecular dynamic simulation (MDS) to understand the mechanisms of action better. Through this process, we uncovered novel phytochemicals that can be potentially employed as Notch inhibitors. Natural phytochemicals derived from plants have been found to be an effective, low-risk treatment for stage 1 breast cancer. Our research presented an in silico workflow for identifying phytochemical compounds as potential sources of anti-cancer medication candidates (Fig. 1).
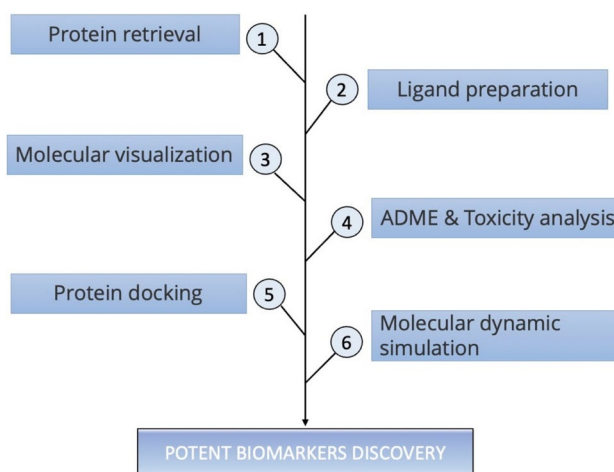


**Fig. 1** Graphical representation of the methods employed in this study

## Materials and methods

### Target protein retrieval

Protein homology models of P53 and NOTCH (1–4) protein sequences (Fig. 2) were retrieved from the Alpha fold homology modelling server (https://alphafold.ebi.ac.uk/). Tumour suppressor protein TP-53 or (P53), neurogenic locus notch homolog protein 1 (NOTCH1), neurogenic locus notch homolog protein 2 (NOTCH2), neurogenic locus notch homolog protein 3 (NOTCH3), and neurogenic locus notch

**Table 1** Phytochemical details of each herb and its anti-cancer activity

| S. no | Phytochemical | Plant Family | Phytochemical family | Anti-cancer activity | Refs |
|---|---|---|---|---|---|
| 1 | Echinacea | Asteraceae | Flavonoids | Increases the natural killer cells | [103] |
| 2 | Garlic | Allium sativum | Sulfurones-Alliin | Interferes with tumour cell metabolism, suppressor T- cells increased | [104] |
| 3 | Turmeric | Curcuma longa | Phenols | Has inhibitory action in all phases of tumour growth | [105] |
| 4 | Burdock | Arctium lappa | Phenols | Stimulates macrophage activity, limits tumour division, preserve immune-modulatory properties | [106] |
| 5 | Green tea | Camellia sinensis | Polyphenols Flavonoids | Restricts cancer cell multiplication, induce apoptosis & necrosis | [107] |
| 6 | Ginseng | Panax ginseng | Ginsenosides | Inhibits the growth and metastasis of malignant cells, increases interferon levels. The natural killer cell cycle is enhanced, promotes the production of antibodies | [108] |
| 7 | Black cohosh | Cimicifuga racemosa | Triterpene glycosides | When combined with chemotherapy drug, has significant impacts on breast tumours | [109] |
| 8 | Flax seed | Linum usitatissimum | Lignans | Decrease of tumour growth | [110] |
| 9 | Ginger | Zingiber officinale | polyphenols | Have antiproliferative effects, proapoptotic | [111] |
| 10 | Black pepper | Piper nigrum | Piperine | High antioxidant properties, interference with signalling pathways | [112] |
| 11 | Black cumin | Nigella sativa | Flavonoid-Thymoquinone | Used as combinational agent, anti-tumour activity | [113] |
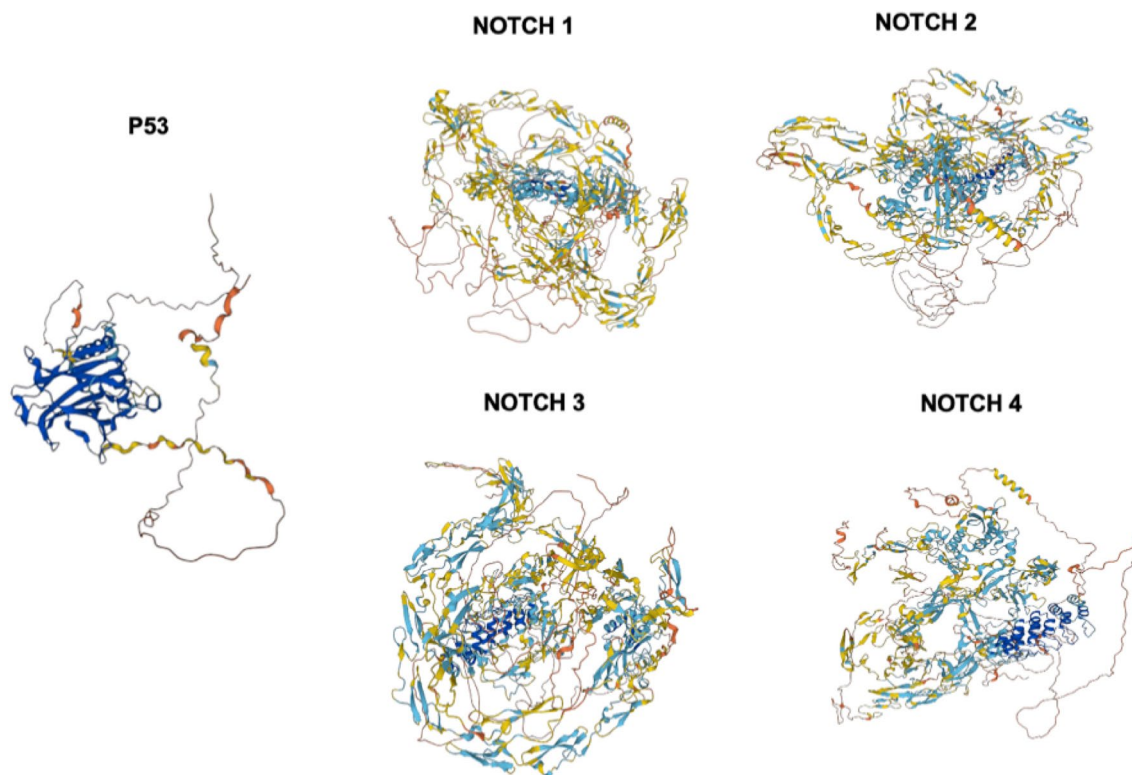
**Fig. 2** Types of proteins involved in Target retrieval. Source: Alpha fold Protein structural database

homolog protein 4 (NOTCH4) were retrieved using the Uni-prot ID P53-P04637, NOTCH1-P46531 NOTCH2-QO4721, NOTCH3-Q9UM47, and NOTCH4-Q99466 [114]. Amino acid (aa) sequences for proteins 393-P53, 2551-NOTCH1, 2471-NOTCH2, 2321-NOTCH3, and 2001-NOTCH4 derived from Homo sapiens (humans) were recovered.

## Preparation of ligands

Research papers based on Ayurveda classical books were scrutinized to compile a comprehensive list of medicinal plants known for vitality and immune enhancing character-istics, which may reflect potential anti-cancerous effects, to guide the selection of herbal bio-compounds [115–120]. Fol-lowing these stipulations, phytochemicals were searched via the IMPPAT, PubChem, and ChEMBL databases, with four herbal phytochemicals shortlisted after a thorough literature survey using google scholar and PubMed databases (Fig. 3) [121, 122]. These ligands were then subject to further molec-ular docking and other downstream analyses. Phytochemi-cal candidates were collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in 3D SDF (Structural data file) format [20, 78, 79]. The files for the protein targets P53 and Notch 1–4 were acquired from the Alpha fold pro-tein structural database. 3D conformers of phytochemicals (Epigallocatechin gallate CID-65064, Piperine CID-638024,
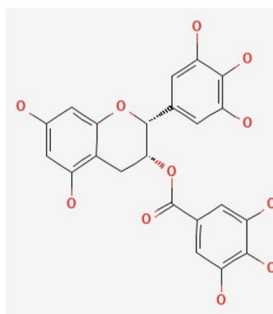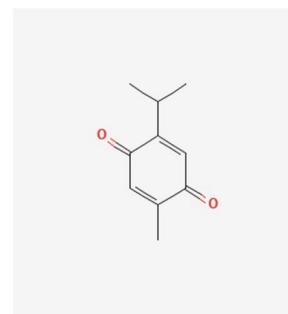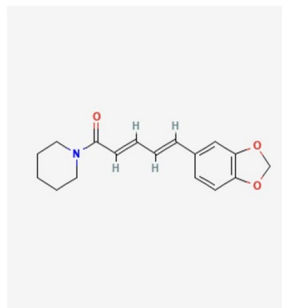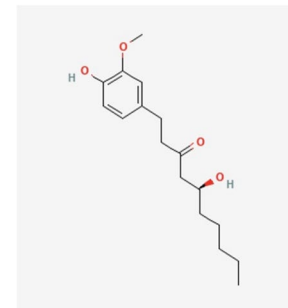


**Fig. 3** Types of herbs and their active bio-compounds 3D conformers retrieved from PubChem database. Source: PubChem database

Gingerol CID-442793, and Thymoquinone CID-10281), henceforth referred to as ligands, were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 14th March 2023) in .SDF format.

## Toxicity assessment

The ADMET server (http://lmmd.ecust.edu.cn/admetsar2) was used to determine the pharmacokinetic parameters as well as the efficacy of various synthetic and phytochemical-based medications. To assist in predicting the ADMET parameters, we also utilized the admetSAR server (http://lmmd.ecust.edu.cn/admetsar2) [123, 124]. The admetSAR server also provided predictions on numerous factors, including toxicity, carcinogenicity, and LD50 values, as well as the Blood–Brain Barrier (BBB), Human Intestinal Absorption (HIA), Caco-2 cell permeability, P-GP (P-glycoprotein) substrate/inhibition, and Cytochrome P450 metabolism.

## Molecular docking study

CB-Dock2, an open-source software (GUI version of Auto Dock Vina), was used to do a virtual screening of herbal plant phytochemicals by molecular docking to the active site of the receptor [124, 125]. This was performed to help screen the optimal inhibitor for P53 and Notch targets. The ligands were matched with each protein individually throughout the docking investigation, with the resulting output files used to extract the binding affinity/Vina score table. The assessment criteria for the best inhibitors predicted were phytochemicals with a stronger affinity for the target protein. Active site prediction was made using PyMol 2.5.2 molecular graphic system software by finding the active sites/binding pockets in the chosen target proteins where the ligand is most likely to bind with stable free energy [125]. PyMol was also used to predict the polar and non-polar interaction analysis of the complexes [126]. To study protein docking, an online-based blind docking server called CB-Dock was used (http://clab.labshare.cn/cb-dock/php/index.php) [127]. Instead of docking blindly throughout a protein's whole surface, CB-Dock was set to only dock at predetermined locations. The initial step was a search for potential binding sites or cavity detection. A few prime cavities were selected for further investigation based on cavity size, as ligand-binding sites are often larger cavities [128]. The docking centre and cavity volume were predetermined based on the cavity pocket (C1 to C5) it was assigned to. The bound poses were reranked based on the vina score after completing the docking process. The best binding site for the query ligand and the first conformation posture based on the highest negative vina score was regarded as the optimum binding posture/conformation. The amino acid residues of each binding conformation was displayed after the docking session is completed.

## Molecular dynamics simulation

Using UCSF CHIMERA 1.17, an extensible molecular modelling system, the best-docked protein target and ligand complexes were refined and put through a molecular dynamics simulation (MDS) study. Protein complexes stored as .pdb complex files were transformed into .psf files and subsequent trajectory files were obtained. These trajectory files were utilized to enhance the complex structures by minimizing solvation and neutralization. A cubic water box with transferrable intermolecular potential was used for the solvation of the complex structures, with the box's dimensions automated. The approximate results were retrieved using generalized born molecular mechanics surface area in an explicit solvent. NVT dynamics were implemented, which maintained constants for substance quantity ($N$), volume ($V$), and temperature ($T$). The complete simulation was run in 1000 steps over 1000 ns with the Noose–Hover temperature set to 300 K. The protein-lipid parameter for proteins and the General Force Field parameter for small-molecule ligands were used as per the study conducted by Santos et al. [129] and they were used as a sample to assign topology and force field parameters [130].

# Results

## ADME and toxicity analysis

The application domain of ADME and Toxicity verification to check the bio-compounds drug likeliness was determined by six physicochemical or topological properties: molecular weight, analogP, number of atoms, number of rings, H-bond acceptors, and H-bond donors [131]. According to Lipinski's Rule of Five, a compound should have threshold values for all six physiochemical properties [132]. The four shortlisted herbal substances underwent pharmacodynamic, physiochemical, absorption, distribution, metabolism, and excretion (ADMET) tests, as indicated in Table 2. Herbal compounds with higher gastrointestinal absorption, solubility, blood–brain barrier permeability, good logP values (partition coefficient), and those that did not break any of Lipinski's Rule of Five (pharmaceutical properties) were listed. Of those that passed the prior parameters, herbal compounds with good bioavailability, lead-likeness scores, and superior synthetic accessibility were then chosen.

Following ADME analysis, phytochemicals were tested for toxicity to select compounds that did not bind to harmful receptors or demonstrate sensitive lethality. Table 3 summarizes the significant toxicity values of each herbal compound. We selected the compounds based on their bioavailability, lead-likeness scores, and easy-to-synthesize properties. After analysing their ADME (Absorption,

**Table 2** Physiochemical properties of herbal compounds provided by admetSAR server

| Active Biocompound | MW | ALogP | WS (logS) | H-Bond acceptor | H-Bond donor | Rotatable bonds | HIA | BBB | Human oral bioavailability (100%) | Plasma protein binding (100%) | Acute oral toxicity Log(1/mol/kg) | Applicability domain |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Epigallocatechin gallate | 458.38 | 2.23 | −3.314 | 11 | 8 | 3 | 0.8422 | 0.8000 | 0.7000 | 0.986 | 1.614 | Warning |
| Piperine | 285.34 | 3.00 | −3.398 | 3 | 0 | 3 | 0.9934 | 0.9250 | 0.5714 | 1.083 | 2.788 | In-domain |
| Gingerol | 294.39 | 3.23 | −3.234 | 4 | 2 | 10 | 0.9911 | 0.6750 | 0.7000 | 0.794 | 1.71 | In-domain |
| Thymoquinone | 164.20 | 1.67 | −2.006 | 2 | 0 | 1 | 0.9969 | 0.7000 | 0.6143 | 0.805 | 1.85 | In-domain |

*MW* molecular weight, *WS* water solubility, *HIA* human intestinal absorption, *BBB* blood–brain barrier

Distribution, Metabolism, and Excretion) characteristics, we tested the chosen phytochemicals to ensure the compounds did not bind to harmful receptors or cause sensitive lethality.

## Interpretation of protein–ligand interaction

Further confirmation via interaction analysis was performed to look for contacts between the docked phytochemicals, Epigallocatechin gallate, Piperine, Gingerol, and Thymoquinone and the target proteins P53 and NOTCH (1–4) before submitting the chosen protein–ligand complexes for MDS study (Fig. 4). To determine how many contacts each phytochemical formed, we treated each of the four phytochemicals to individual protein pair docking with P53 and NOTCH (1–4). In our interaction study, we observed that complexes with strong binding affinities were those that produced the most hydrogen bonds, as shown in Fig. 4.

The unique binding characteristics of each compound was thoroughly investigated with respect to the individual receptors. To elucidate the differentiation in binding mode with the Notch 1–4 receptors, the extensive molecular docking analysis and molecular dynamics simulations study would provide us with the report of the binding modes. Our findings reveal distinct interaction patterns between each compound and the receptors, contributing to their selective binding.

1. Epigallocatechin Gallate (Green Tea)

   - **Notch 1:** Epigallocatechin gallate interacts with Notch 1 through a combination of hydrogen bonding and hydrophobic interactions. Within the binding pocket of Notch 1, its hydroxyl groups engage in hydrogen bonding with specific amino acid residues, such as asparagine and serine. These interactions stabilize the complex and contribute to binding affinity. Additionally, the hydrophobic regions of Epigallocatechin gallate's aromatic rings align with hydrophobic residues in the binding pocket, further enhancing its binding specificity to Notch 1.
   - **Notch 2:** Epigallocatechin gallate binding to Notch 2 involves a distinct set of interactions. In this case, galloyl groups form hydrogen bonds with polar residues within the binding site of Notch 2, such as threonine and glutamine. The unique arrangement of phenolic rings allow for π–π stacking interactions with specific aromatic residues in Notch 2. These interactions contribute to the compound's orientation and binding stability with this receptor.
   - **Notch 3:** Binding to Notch 3 is characterized by a combination of hydrogen bonding and van der Waals interactions. Epigallocatechin gallate's hydroxyl groups form hydrogen bonds with key residues in

**Table 3** (a) Toxicity prediction of selected herbal compounds. (b) Acute toxicity category values from the admetSAR server

**(A) Toxicity prediction of selected herbal compounds**

| Phytochemicals | AMES Toxicity | Carcinogens | Acute oral toxicity | Nephrotoxicity | Biodegradability | BRCP-Breast cancer receptor protein |
|---|---|---|---|---|---|---|
| Epigallocatechin gallate | AMES nontoxic | Non-carcinogenic | IV | Negative | Biodegradable | Negative |
| Piperine | AMES nontoxic | Non-carcinogenic | III | Negative | Biodegradable | Negative |
| Gingerol | AMES nontoxic | Non-carcinogenic | III | Negative | Biodegradable | Negative |
| Thymoquinone | AMES nontoxic | Non-carcinogenic | II | Positive | Biodegradable | Negative |

**(B) Acute toxicity category values from the admetSAR server**

| Acute Toxicity | Category I | Category II | Category III | Category IV | Category V |
|---|---|---|---|---|---|
| Oral (mg/kg) | ≤ 5 (Very low) | > 5 to ≤ 50 (medium) | > 50 to ≤ 300 (medium) | > 300 to ≤ 2000 (very high) | 2000 to 5000 mg/kg |
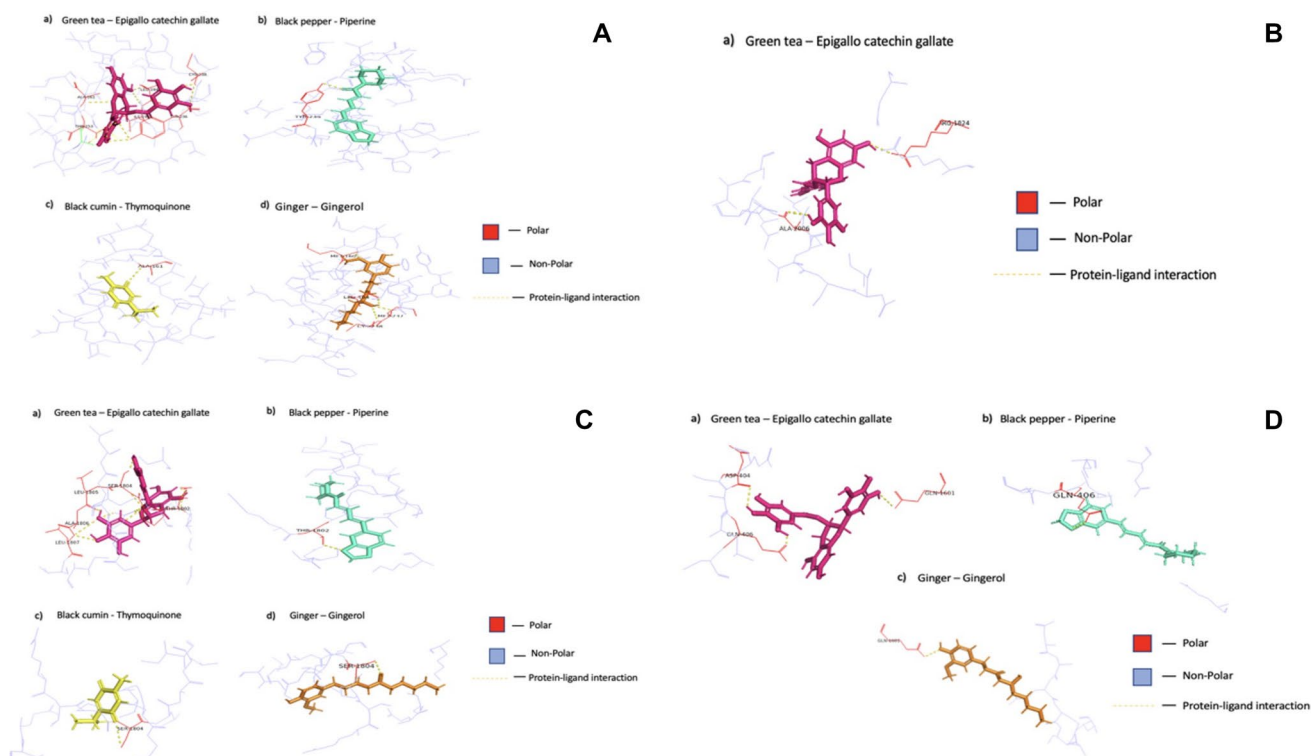


**Fig. 4** **A** Binding site residue of P53 protein with phytochemicals, **B** NOTCH1 Protein, **C** NOTCH2 protein, and **D** NOTCH4 protein

the binding pocket, including histidine and arginine. Furthermore, flexible molecular structure enables it to fit snugly within the binding site, forming van der Waals contacts with hydrophobic residues. This arrangement optimizes the binding affinity of Epigallocatechin gallate with Notch 3.

- **Notch 4:** Epigallocatechin gallate's binding to Notch 4 is mediated by a mix of hydrogen bonding and electrostatic interactions. The galloyl groups

engage in hydrogen bonds with specific polar residues in the binding pocket, such as tyrosine and lysine. The distribution of charges in structure complements the electrostatic environment of Notch 4's binding site, further enhancing its binding specificity. Hydrophobic interactions between aromatic rings and hydrophobic residues contribute to the stabilization of the complex.

2.  Piperine (Black Pepper)

- **Notch 1:** Piperine could potentially interact with Notch 1 through a combination of hydrogen bonding and hydrophobic interactions. Its aromatic ring structures might align with hydrophobic residues in the Notch 1 binding pocket, whilst functional groups on Piperine, such as hydroxyl or amine groups, could form hydrogen bonds with specific amino acid residues in the receptor.
- **Notch 2:** For binding to Notch 2, Piperine might form specific hydrogen bonds with polar residues within the binding site, allowing it to orient itself optimally. Additionally, its flexible molecular structure might enable it to engage in π–π stacking interactions with aromatic residues in Notch 2, contributing to its binding affinity.
- **Notch 3:** Piperine's interaction with Notch 3 could involve a mix of hydrogen bonding and van der Waals interactions. Its functional groups might form hydrogen bonds with key polar residues in the binding pocket, whilst its non-polar regions could engage in van der Waals contacts with hydrophobic residues.
- **Notch 4:** Piperine's binding to Notch 4 could rely on hydrogen bonding and electrostatic interactions. Its charged or polar groups might form hydrogen bonds with specific residues in the binding pocket and its distribution of charges could complement the electrostatic environment of Notch 4.

3. Gingerol (Ginger)

- **Notch 1:** Gingerol could potentially interact with Notch 1 through a combination of hydrogen bonding and hydrophobic interactions. Its aromatic ring structures might align with hydrophobic residues in the Notch 1 binding pocket, whilst functional groups on Gingerol, such as hydroxyl or methoxy groups, could form hydrogen bonds with specific amino acid residues in the receptor.
- **Notch 2:** For binding to Notch 2, Gingerol might form specific hydrogen bonds with polar residues within the binding site, allowing it to orient itself optimally. Its flexible molecular structure might enable it to fit into the binding pocket and establish interactions with residues critical for Notch 2 binding.
- **Notch 3:** Gingerol's interaction with Notch 3 could involve a mix of hydrogen bonding and van der Waals interactions. Its functional groups might form hydrogen bonds with key polar residues in the binding pocket and its non-polar regions could engage in van der Waals contacts with hydrophobic residues.
- **Notch 4:** Gingerol's binding to Notch 4 could rely on hydrogen bonding and electrostatic interactions. Its charged or polar groups might form hydrogen bonds

with specific residues in the binding pocket and its distribution of charges could complement the electrostatic environment of Notch 4.

4. Thymoquinone (Black Cumin)

- **Notch 1:** Thymoquinone could potentially interact with Notch 1 through a combination of hydrogen bonding and hydrophobic interactions. Its aromatic ring structures might align with hydrophobic residues in the Notch 1 binding pocket, while functional groups on thymoquinone, such as hydroxyl or carbonyl groups, could form hydrogen bonds with specific amino acid residues in the receptor.
- **Notch 2:** For binding to Notch 2, Thymoquinone might form specific hydrogen bonds with polar residues within the binding site, allowing it to orient itself optimally. Its molecular structure might enable it to engage in π–π stacking interactions with aromatic residues in Notch 2, contributing to its binding affinity.
- **Notch 3:** Thymoquinone's interaction with Notch 3 could involve a mix of hydrogen bonding and van der Waals interactions. Its functional groups might form hydrogen bonds with key polar residues in the binding pocket and its non-polar regions could engage in van der Waals contacts with hydrophobic residues.
- **Notch 4:** Thymoquinone's binding to Notch 4 could rely on hydrogen bonding and electrostatic interactions. Its charged or polar groups might form hydrogen bonds with specific residues in the binding pocket and its distribution of charges could complement the electrostatic environment of Notch 4.

Specific binding interactions between Piperine and Notch receptors have not been extensively studied or reported in the scientific literature. However, explanations based on general molecular interactions can be given. Research on Piperine binding has been limited, thus we aimed to explore how Piperine might differentiate in binding mode with Notch receptors.

The binding interactions between the ligands and the proteins visualized via PyMol Visualizer Tool showed better interaction with the P53 Protein (Uniprot ID- P04637). P53-Green tea shows polar contact with the ligand at ALA161, LEU194, SER-215, THR253, CYS238, TYR-236, and ILE-19, whereas P53-Black pepper interaction yielded contact at TYR-236, P53-Black cumin at ALA161, and P53-Ginger interaction shows contact at MET-160, LEU194, MET-237, and CYS238. Protein–ligand polar contact with amino acids was observed with the NOTCH2 protein, but no hydrogen bonds were visible. NOTCH2-Green tea non-polar contacts were seen in amino acids THR-119 and THR-121. NOTCH2-Black pepper pair amino acids were THR-121,

CYS-120, and THR-119. NOTCH2-Black cumin pair and their non-polar amino acids were THR-121 and THR-119. NOTCH2-Ginger pair showed contact signs at THR-119, CYS-120, and THR-121. NOTCH3 protein did not have any protein–ligand interaction, which was consistent with other analyses in the current literature. NOTCH4-Green tea complex showed hydrogen bonds at sites ASP404, GLN406, and GLN1601. NOTCH4-Black pepper yielded contact in GLN406, and NOTCH4-Ginger contact was seen in GLN1601. The consolidated data for the amino acid residues are presented in Table 4. Since these are known to stabilize protein complexes and are important for molecular recognition processes, Epigallocatechin gallate was notable for producing many hydrogen bonds. Due to the conformational space that Piperine, Gingerol, and Thymoquinone inhabited, they produced a few hydrogen bonds. The interactions between the target proteins P53 and NOTCH (1–4) and the shortlisted ligands are shown in Fig. 4.

It is important to note that these explanations are speculative and based on general knowledge of molecular interactions. The actual binding modes of Piperine, Gingerol, and Thymoquinone with Notch receptors would require rigorous experimental investigation, such as molecular docking studies, X-ray crystallography, or NMR spectroscopy, to provide accurate details about the binding interactions. In summary, our computational analyses have revealed that the compounds exhibit varying binding modes with the Notch 1–4 receptors due to their distinct chemical structures and interactions. We believe these findings shed light on the molecular basis of the compounds' selectivity and efficacy towards different Notch receptors.

## Protein docking

Four phytochemicals examined after the docking study with higher affinity/scoring function for the target proteins NOTCH 1–4 and P53 are shown in Table 3 as Epigallocatechin gallate, Piperine, Gingerol, and Thymoquinone. The phytochemicals docked to the target proteins NOTCH 1–4 and P53 as shown in Fig. 5. Our investigation into the effects of certain compounds on the Notch receptors has yielded intriguing results that could pave the way for novel therapeutic strategies. For Epigallocatechin gallate the binding pockets of Notch 1, 2, 3, and 4 form many hydrogen bonds and hydrophobic interactions with certain residues, according to our computational investigations. Notably, aromatic rings form van der Waals interactions with hydrophobic residues, whereas its hydroxyl groups form hydrogen bonds with polar amino acids. According to this binding method, Epigallocatechin gallate may interfere with crucial cleavage events and impede ligand–receptor interactions, affecting each Notch receptor's downstream signalling cascades (refer to Fig. 5). Piperine, for instance, has been found to adopt distinct binding poses in the binding sites of Notch 1, 2, 3, and 4 (In supplementary Figs. 1–4). This is due to its flexible structure, which allows it to establish hydrogen bonds with specific polar residues and participate in π–π stacking interactions with key aromatic amino acids. The implications of these interactions are manifold; they suggest that Piperine has the potential to not just interfere with ligand binding but also cleavage events or downstream signalling processes for each Notch receptor. Similarly, our analysis of Gingerol has revealed that it interacts with the Notch receptors through a combination of hydrogen bonds and hydrophobic contacts (In supplementary Figs. 1–4). Gingerol's binding poses suggest it may interfere with ligand recognition, cleavage, or nuclear complex formation. The interactions that Gingerol has with specific residues in the binding pockets of each Notch receptor also suggest its ability to modulate key signalling events. Finally, Thymoquinone has been found to establish binding interactions involving hydrogen bonding, electrostatic interactions, and van der Waals forces with

**Table 4** Consolidated results for protein–ligand interaction

| Protein–Ligand Complex | Hydrogen bond residues |
|---|---|
| P53—Green tea | ALA161, LEU194, CYS238, TYR236, ILE195, THR253 |
| P53—Black pepper | TYR236 |
| P53—Ginger | MET-160, LEU194, CYS238, MET-237 |
| P53—Black cumin | ALA161 |
| Notch 1—Green tea | ALA2006, ARG 1824 |
| Notch 2—Green tea | LEU1807, ALA1806, LEU1805, SER1804, THR1802 |
| Notch 2—Black pepper | THR1802 |
| Notch 2—Black cumin | SER1804 |
| Notch 2—Ginger | SER1804 |
| Notch 4—Green tea | ASP404, GLN406, GLN1601 |
| Notch 4—Black pepper | GLN406 |
| Notch 4—Ginger | GLN1601 |

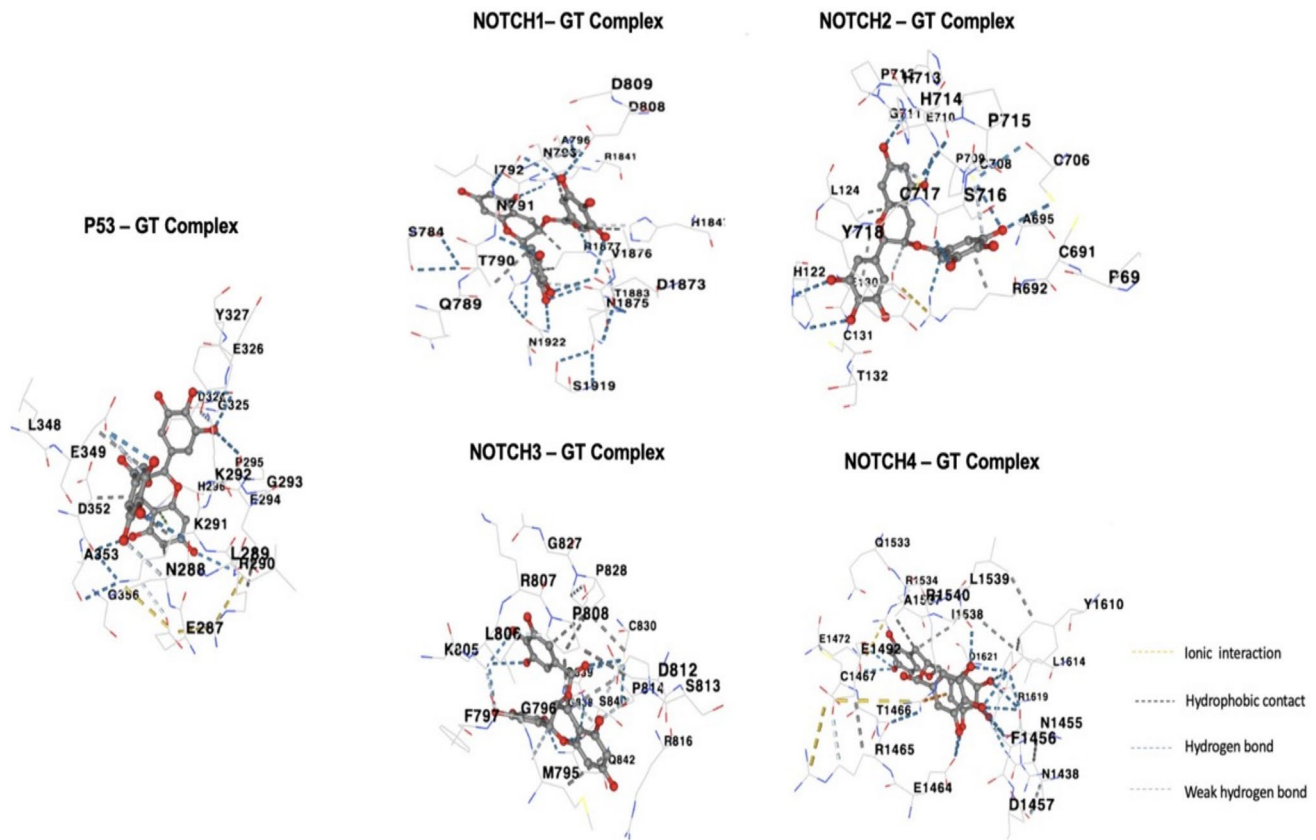*Notch 3 proteins had no bonds involved with the phytochemicals

**Fig. 5** Docked poses of Green tea ligand Epigallocatechin gallate with P53 and NOTCH 1–4 proteins. Yellow-dotted lines represent Ionic/electrostatic interactions, teal-dotted lines represent strong hydrogen bonds, whilst hydrophobic interactions are represented by grey-dotted lines

Notch 1, 2, 3, and 4 (In supplementary Figs. 1–4). These interactions suggest that Thymoquinone may influence ligand binding, cleavage, or other crucial steps in Notch signalling. The variations in binding modes across the receptors offer insights into its potential selectivity. Taken together, these findings provide a detailed understanding of how certain compounds can interact with the Notch receptors and offer new avenues for research into the development of targeted therapies.

The Epigallocatechin gallate ligand appeared to have the best docking results via MDS based on the Vina score of all tested bio-compounds. Docking results of the compounds (Epigallocatechin gallate, Piperine, Gingerol, Thymoquinone) with the P53 and NOTCH (1–4) proteins showed a ball and stick representation of the ligands, with cartoon backbone representation of target proteins displayed in Fig. 5. The overall docked score for each pair is shown in Supplementary Table 1 and 2, which gave information on cavity size, cavity volume, centre (x,y,z) axis, amino acid residue numbers, and docked vina score that provided in-depth information about each protein–ligand pair and their corresponding figures as shown in Supplementary Figs. 1–5. As the Epigallocatechin gallate (Green tea) ligand showed

the highest scoring function, we performed further CB-dock experiments with the target proteins to analyse the various protein–ligand complexes (Table 5).

## Molecular dynamics simulation

UCSF Chimera was used to conduct a molecular dynamic simulation (MDS) study to understand the conformational changes that occur over time, along with the mode of interface with the receptor and its ligand. We chose one phytochemical receptor complex based on the highest scoring function and ran a 2500-step MDS on them for 100 ns. Next, using the TIP3PBOX solvate water model in a cubic box with box size 1, the system was set automatically for runs, and when all the systems were neutralized with the addition of Cl ions, energy minimization was done to eliminate any steric incompatibilities within the system. After that, a 1-nano second NVT and NPT simulation was run to keep the system's pressure at 1 atm and temperature at 300 K. The complexes were then put forward for a 100-ns MDS, and the coordinates were saved once per femtosecond. The Root Mean Square Deviation (RMSD) plot for each protein–ligand complex was analysed following

**Table 5** Results of CB-dock of green tea ligand with target proteins (P53, NOTCH 1–4)

| S. No | Target | Ligand | Vina score | Curpocket ID | Cavity volume (A°3) | Centre | Contact residues |
|---|---|---|---|---|---|---|---|
| 1 | P53 | Green tea | −9.2 | C1 | 1146 | −8,16, −26 | GLU287 ASN288 LEU289 ARG290 LYS291 LYS292 GLY293 GLU294 PRO295 HIS296 HIS297 ASP324 GLY325 GLU326 TYR327 LEU348 GLU349 ASP352 ALA353 GLY356 |
| 2 | NOTCH 1 | Green tea | −8.1 | C1 | 4802 | 28, −2,18 | SER784 GLN789 THR790 ASN791 ILE792 ASN793 ALA796 ASP808 ASP809 ARG1841 HIS1847 ASP1873 ASN1875 VAL1876 ARG1877 THR1883 SER1919 ASN1922 |
| 3 | NOTCH 2 | Green tea | −8.1 | C2 | 1661 | −14, 34, −24 | HIS122 LEU124 GLU130 CYS131 THR132 PRO690 CYS691 ARG692 ALA695 CYS706 CYS708 PRO709 GLU710 GLY711 PRO712 HIS713 HIS714 PRO715 SER716 CYS717 TYR718 |
| 4 | NOTCH 3 | Green tea | −9.4 | C3 | 1502 | 44, −7, −8 | ASN1438 ASN1455 PHE1456 ASP1457 GLU1464 ARG1465 THR1466 CYS1467 GLU1472 GLU1492 GLN1533 ARG1534 ALA1537 ILE1538 LEU1539 ARG1540 TYR1610 LEU1614 ARG1619 ASP1621 |
| 5 | NOTCH 4 | Green tea | −7.5 | C1 | 1084 | −23, −39, 22 | MET795 GLY796 PHE797 LYS805 LEU806 ARG807 PRO808 ASP812 SER813 PRO814 ARG816 GLN826 GLY827 PRO828 CYS830 GLY838 GLY839 SER840 GLN842 |

the simulation. Results of the MDS study and refinement process indicated that the complexes had been optimized for refinement, and the overall energy of the complex has also been stabilized, as structures presented good RMSD scores.

From our observations, Epigallocatechin gallate with Notch 3 protein appeared to be the most stable complex due to an observed higher overall energy than the other tested phytochemicals. The stability of the refined structure was encapsulated by the RMSD, which showed various oscillations present in the complex during the MDS and checked to see if the simulation had been balanced. The contacts and ligand-binding posture energy variability values of the Green tea ligand bound to Notch 3 was reported to have an RMSD score of 0.66, indicating minor changes following the refining of the complex. The molecular dynamic study was separately performed for Epigallo-catechin gallate ligand alone as seen in Fig. 6 and with the NOTCH3 protein complex as shown in Fig. 7. The results showed that in different time frames, the RMSD value tended to change indicating the phytochemical's stability in the complex. When the ligand was tested separately, the RMSD values of each time frame were mostly within the acceptable range (with the exception of a few outliers that exceeded the threshold value). However, the RMSD values were all within the acceptable range in the protein–ligand complex. This indicated that the complex was likely to be stable and could be used as a basis for further wet-lab experiments (Fig. 7).

## Discussion and future scope

Drug discovery and development are a lengthy, expensive, and risky process that can take up to 10–15 years to complete. On average, each new drug that's approved for clinical use costs over $1–2 billion to develop and 90% of drug development ventures often lead to clinical failures [133]. According to clinical trial data from 2010 to 2017, the main reasons for these failures were lack of clinical efficacy, unmanageable toxicity, poor therapeutic properties, limited commercial needs, and poor strategic planning [91, 92]. Early assessment of a compound's pharmaceutical properties is crucial for improving the effectiveness of the research and development process[134, 135]. However, the conventional methods currently used to assess ADMET characteristics are costly, time-consuming, and typically need substantial animal experimentation [136]. The preferred method for early drug discovery is now based on computer modelling approaches for predictive models [137]. Analysing a compound's ADMET qualities in the early stages of drug development allows for the preliminary elimination of compounds with unfavourable ADME-Tox properties. In addition to using the predictive models as a guide for hit/lead compounds, the identification and structural optimisation of the compound can also yield extensive information that further elucidates its pharmaceutical and chemical properties. Existing in silico, in vitro, and in vivo ADME-Toxicity data should be supported by intelligent integration in the era of big data to better direct drug discovery pathways.

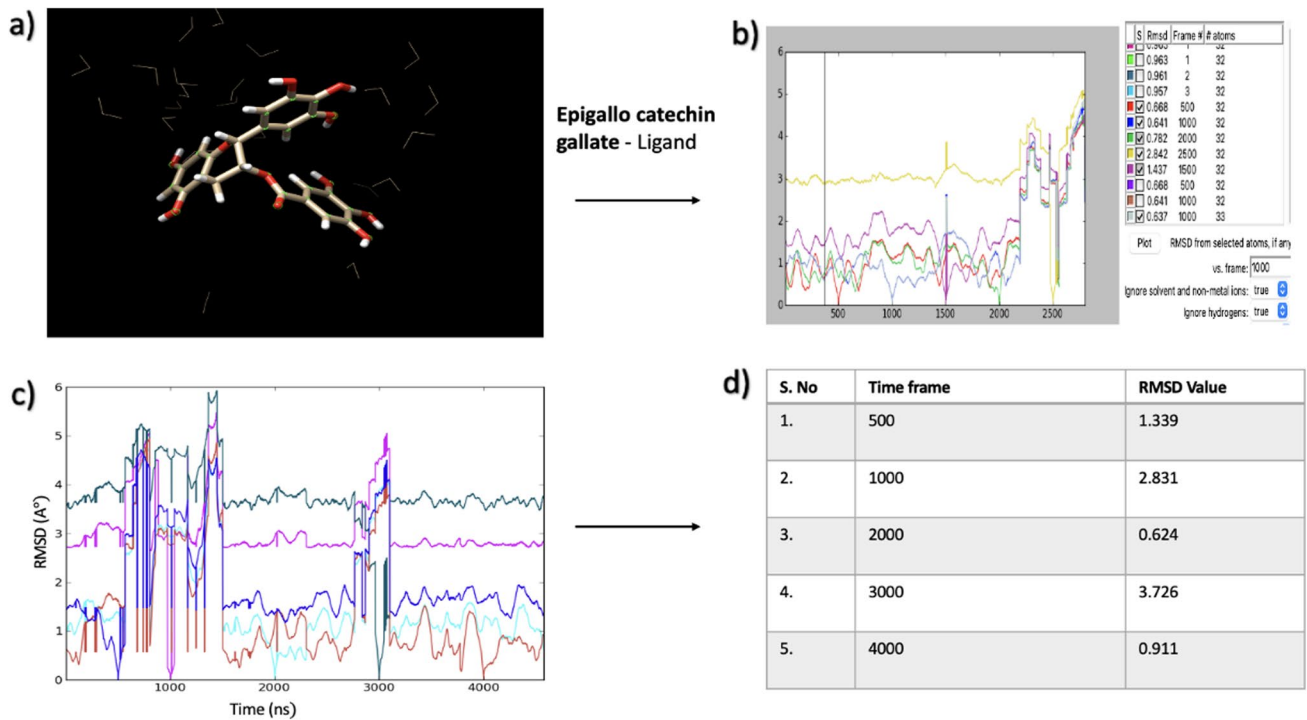Fig. 6 **a** Green tea active bio-compound Epigallocatechin gallate during MDS study. **b** RMSD plot filters based on time frame. **c** Root mean square deviation (RMSD) plot for ligand structure after three steps (Minimization, equilibration, production)
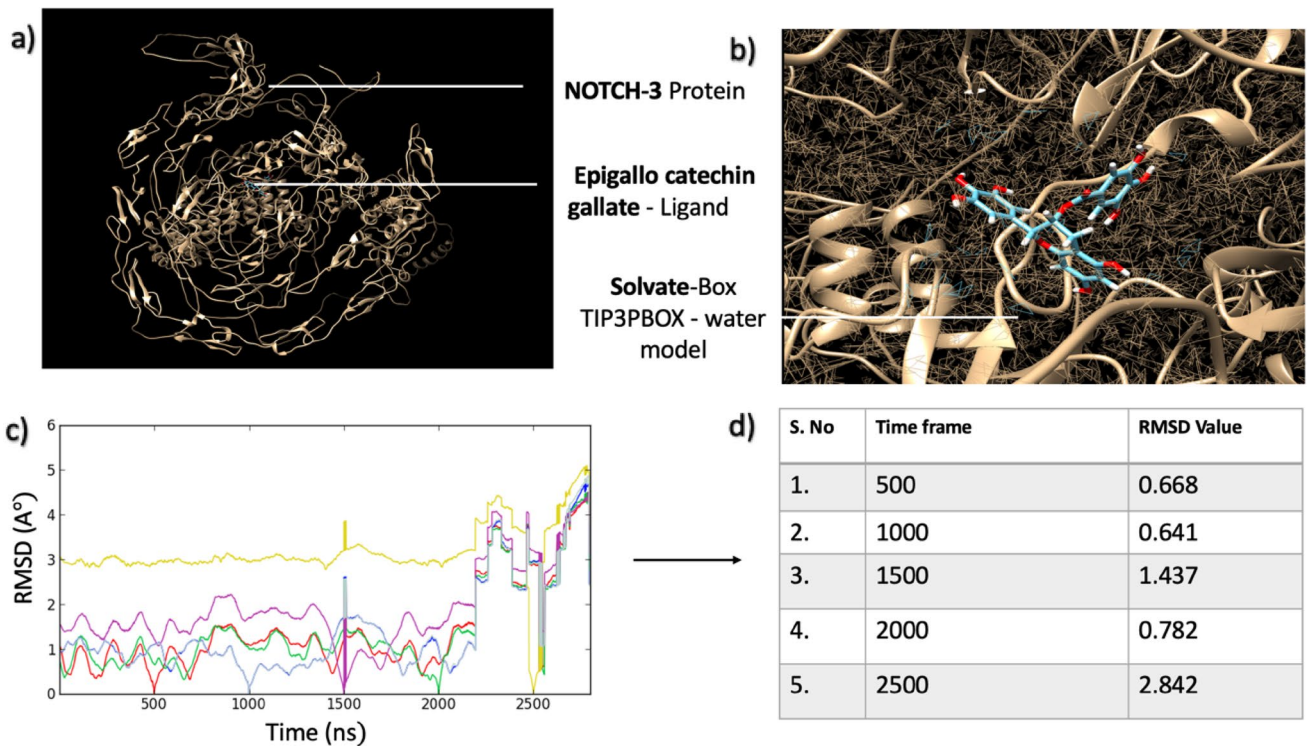


Fig. 7 **a** NOTCH3 protein-Green tea active bio-compound Epigallocatechin gallate before MDS study, **b** Target-ligand complex during minimization process, **c** Root mean square deviation (RMSD) plot for target-ligand complex after four steps (Minimization, equilibration, production)

The interaction between protein and ligand is crucial in the design of drugs. Studies of protein–ligand interactions offer a theoretical framework for developing and identifying novel therapeutic targets [138, 139]. The interactions between Notch receptors and plant-derived ligands explored in this study have provided promising computational information on candidate compounds for use in breast cancer therapy. This includes developing a framework for comprehensive analyses of the mechanism of interactions, signalling, and binding affinity between phytochemicals and Notch Receptors via computational techniques. Our main work was to examine herbal phytochemicals that may theoretically and effectively interact with the NOTCH 1,2,3,4, and P53 proteins, increasing cancer cell death and reducing tumour spread against breast cancer cells. Only 4 of the 12 ADMET-screened compounds (Epigallocatechin gallate, Piperine, Gingerol, Thymoquinone) were found to be viable, and these were the ones that underwent molecular docking. Several of these phytochemicals were excluded due to a lack of .sdf/.mol structures available, or parsing errors occurred when they were subjected to molecular docking research. Thus, only four of the phytochemicals shortlisted were used for the molecular docking analysis.

The highest negative scoring function (higher negative vina score/binding energy) appeared to be the ideal preference as it showed better binding affinity between protein–ligand pairs [96, 97]. The binding energy discovered in the phytochemicals explored in this study was between $-9.4$ and $-5.1$ kcal/mol, which agreed with the ideal and standard binding energies. Our analysis revealed that ALA161, LEU194, CYS238, TYR236, ILE195, THR253 in P53 protein, along with ALA2006 and ARG 1824 in Notch 1 protein, LEU1807, ALA1806, LEU1805, SER1804, and THR1802 in Notch 2 protein, ASP404, GLN406, and GLN1601 in Notch 4 protein were promising residues for therapeutic targets. In addition, Epigallocatechin gallate, a ligand derived from green tea, showed potential as a compound for inhibiting the Notch 3 Receptor. Although the Notch 3 protein did not appear to interact with the Epigallocatechin gallate ligand, in silico docking analyses showed a high docking score/binding affinity, meaning further protein–ligand complex validation appears promising for potential therapeutic discovery [140, 141].

Creating therapeutic lead molecules using natural bioproducts through in silico modelling could save substantial time in creating new therapeutic products [142]. Clinical studies and wet-lab experimentation for validation are the logical next step in establishing these phytochemicals as new avenues of treatment for breast cancer and other cancers in general. Future drug development processes will take less time and resources due to the commercial computational tools that are readily available for calculating the pharmacological drug properties of candidate molecules. Findings from extensive in silico workflows will be important in selecting lead phytoconstituents for additional drug discovery steps. Within the current literature, the interest in herbal therapeutic compounds with the potential to provide resistance to breast cancer in humans is increasing. Herbal compounds with medicinal properties have been widely utilized for treatments of various diseases, such as diabetes, blood pressure, cancer, heart-related ailments, neurodegenerative diseases, and gastric issues [143]. There is growing evidence that herbally derived compounds provide a promising approach towards preventing and inhibiting breast cancer [144]. To advance our knowledge of using natural herbal compounds in breast cancer treatment and prevention, more research is required to explore the potential anti-breast cancer effects of these various compounds.

This study aimed to identify safe and efficient phytochemicals with the potential to work alongside signalling proteins to disrupt the cancer cycle. By screening several phytochemicals derived from Indian herbs, the in silico workflow developed in the study was able to uncover lead compounds for breast cancer therapy. Four phytochemicals, namely Epigallocatechin gallate, Piperine, Gingerol, and Thymoquinone, showed substantial binding with P53 and NOTCH proteins. These phytochemicals are also found in various other Indian herbs in combinational form, which presents the potential for further discovery. The current in silico investigation for putative P53 and NOTCH protein inhibitors from Indian herbs presented novel targets for the interaction with breast cancer biomarkers. This has the potential to direct further clinical research into the safe and efficient development of anti-cancer therapies.

**Author contributions** NS conceptualized the study and performed the analyses with supervision from PV and PK. NS and PV wrote the manuscript with input from PK. All authors read and approved the final version of the manuscript.

**Data availability** The authors confirm that the data supporting this study's findings is either available within the article or can be shared on reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

## References

1. Burstein HJ et al (2021) Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 32(10):1216–1235. https://doi.org/10.1016/j.annonc.2021.06.023

2. 'Breast cancer'. https://www.who.int/news-room/fact-sheets/detail/breast-cancer . Accessed 25 May 2023

3. Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics, 2022. CA: Cancer J Clin 72(1):7–33. https://doi.org/10.3322/caac.21708

4. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer statistics, 2021. CA: Cancer J Clin 71(1):7–33. https://doi.org/10.3322/caac.21654

5. 'Breast cancer|Elsevier Enhanced Reader'. https://reader.elsevier.com/reader/sd/pii/S0140673620323813?token=F04EE001F4AFA148D57A6A18E312759B1B7668E8D437C2F0884D470AD396C5CE814A78A340AB299BD62E2935A33FB6C4&originRegion=us-east-1&originCreation=20230323035152. Accessed 23 Mar 2023

6. 'Adjuvant palbociclib for early breast cancer: the PALLAS trial results (ABCSG-42/AFT-05/BIG-14–03)|J Clin Oncol. https://ascopubs.org/doi/10.1200/JCO.21.02554. Accessed 23 Mar 2023

7. Wani AK et al (2023) Targeting apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. Biomolecules 13(2):194. https://doi.org/10.3390/biom13020194

8. Basak D, Arrighi S, Darwiche Y, Deb S (2021) Comparison of anticancer drug toxicities: paradigm shift in adverse effect profile. Life (Basel) 12(1):48. https://doi.org/10.3390/life12010048

9. Peddie N, Agnew S, Crawford M, Dixon D, MacPherson I, Fleming L (2021) The impact of medication side effects on adherence and persistence to hormone therapy in breast cancer survivors: a qualitative systematic review and thematic synthesis. Breast 58:147–159. https://doi.org/10.1016/j.breast.2021.05.005

10. Sharmin S et al (2021) Cytotoxicity of synthetic derivatives against breast cancer and multi-drug resistant breast cancer cell lines: a literature-based perspective study. Cancer Cell Int 21(1):612. https://doi.org/10.1186/s12935-021-02309-9

11. Fatma H, Siddique HR (2023) Research and patents status of selected phytochemicals against cancer: how close and how far? Recent Pat Anti-Cancer Drug Discov 18(4):428–447. https://doi.org/10.2174/1574892818666221107113648

12. Mandal MK, Mohammad M, Parvin SI, Islam MM, Gazi HAR (2023) A short review on anticancer phytochemicals. Pharmacogn Rev. https://doi.org/10.5530/097627870236

13. Wang J et al (2011) Adjuvant chemotherapy and radiotherapy in triple-negative breast carcinoma: a prospective randomized controlled multi-center trial. Radiother Oncol 100(2):200–204. https://doi.org/10.1016/j.radonc.2011.07.007

14. Buick DL, Petrie KJ, Booth R, Probert J, Benjamin C, Harvey V (2000) Emotional and functional impact of radiotherapy and chemotherapy on patients with primary breast cancer. J Psychosoc Oncol 18(1):39–62. https://doi.org/10.1300/J077v18n01_03

15. Rosental B, Appel MY, Yossef R, Hadad U, Brusilovsky M, Porgador A (2012) The effect of chemotherapy/radiotherapy on cancerous pattern recognition by NK cells. Curr Med Chem 19(12):1780–1791. https://doi.org/10.2174/092986712800099730

16. Fatma H, Jameel M, Siddique HR (2023) An update on phytochemicals in redox homeostasis: "virtuous or evil" in cancer chemoprevention? Chemistry 5(1):201–222. https://doi.org/10.3390/chemistry5010017

17. Akhtar MF, Saleem A, Rasul A, Faran Ashraf Baig MM, BinJumah M, Abdel Daim MM (2020) Anticancer natural medicines: an overview of cell signaling and other targets of anticancer phytochemicals. Eur J Pharmacol 888:173488. https://doi.org/10.1016/j.ejphar.2020.173488

18. Chirumbolo S, Bjørklund G, Lysiuk R, Vella A, Lenchyk L, Upyr T (2018) Targeting cancer with phytochemicals via their fine tuning of the cell survival signaling pathways. Int J Mol Sci 19(11):3568. https://doi.org/10.3390/ijms19113568

19. Dandawate PR, Subramaniam D, Jensen RA, Anant S (2016) Targeting cancer stem cells and signaling pathways by phytochemicals: novel approach for breast cancer therapy. Semin Cancer Biol 40–41:192–208. https://doi.org/10.1016/j.semcancer.2016.09.001

20. PubChem, 'PubChem'. https://pubchem.ncbi.nlm.nih.gov/. Accessed 14 Apr 2023

21. Chen H, Liu RH (2018) Potential mechanisms of action of dietary phytochemicals for cancer prevention by targeting cellular signaling transduction pathways. J Agric Food Chem 66(13):3260–3276. https://doi.org/10.1021/acs.jafc.7b04975

22. Jit BP et al (2022) Phytochemicals: potential therapeutic modulators of radiation induced signaling pathways. Antioxidants 11(1):49. https://doi.org/10.3390/antiox11010049

23. 'Full article: Phytochemicals modulate carcinogenic signaling pathways in breast and hormone-related cancers'. https://www.tandfonline.com/doi/full/10.2147/OTT.S83597. Accessed 26 May 2023

24. Dong L, He J, Luo L, Wang K (2023) Targeting the interplay of autophagy and ROS for cancer therapy: an updated overview on phytochemicals. Pharmaceuticals 16(1):92. https://doi.org/10.3390/ph16010092

25. Siebel C, Lendahl U (2017) Notch signaling in development, tissue homeostasis, and disease. Physiol Rev 97(4):1235–1294. https://doi.org/10.1152/physrev.00005.2017

26. You W-K, Schuetz TJ, Lee SH (2023) Targeting the DLL/Notch signaling pathway in cancer: challenges and advances in clinical development. Mol Cancer Ther 22(1):3–11. https://doi.org/10.1158/1535-7163.MCT-22-0243

27. Qiao L, Wong BCY (2009) Role of Notch signaling in colorectal cancer. Carcinogenesis 30(12):1979–1986. https://doi.org/10.1093/carcin/bgp236

28. 'Mammary stem cells and breast cancer: role of Notch signalling | SpringerLink'. https://doi.org/10.1007/s12015-007-0023-5. Accessed 26 May 2023

29. 'Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance | Nature Immunology'. https://www.nature.com/articles/ni1164. Accessed 26 May 2023

30. Pellegrinet L et al (2011) Dll1- and Dll4-mediated notch signaling are required for homeostasis of intestinal stem cells.

Gastroenterology 140(4):1230-1240.e7. https://doi.org/10.1053/j.gastro.2011.01.005

31. Zardawi SJ, O'Toole SA, Sutherland RL, Musgrove EA (2009) Dysregulation of hedgehog, Wnt and Notch signalling pathways in breast cancer. Histology and histopathology. https://digitum.um.es/digitum/handle/10201/35991. Accessed 26 May 2023

32. 'Full article: Advancements of compounds targeting Wnt and Notch signalling pathways in the treatment of inflammatory bowel disease and colon cancer'. https://doi.org/10.1080/1061186X.2020.1864741?casa_token=0ZiYfgWwTSMAAAAA%3Ah_lZHyVQ8fYObxRaxsipdXp4xjkGU4gduoGLjmkekOmR7d5BBaMHAjn5URj7X8TIfW4P8P1iKr_hMA. Accessed 26 May 2023

33. 'Inflammation and Notch signaling: a crosstalk with opposite effects on tumorigenesis | Cell Death & Disease'. https://www.nature.com/articles/cddis2016408. Accessed 26 May 2023

34. 'Hoek: Expression profiling reveals novel pathways... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Expression%20profiling%20reveals%20novel%20pathways%20in%20the%20transformation%20of%20melanocytes%20to%20melanomas. Accessed 29 Mar 2023

35. 'Liu: Notch1 signaling promotes primary melanoma progressi... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Notch1%20signaling%20promotes%20primary%20melanoma%20progression%20by%20activating%20mitogen-activated%20protein%20kinase/phosphatidylinositol%203-kinase-Akt%20pathways%20and%20up-regulating%20N-cadherin%20expression. Accessed 29 Mar 2023

36. 'Hallahan: The SmoA1 mouse model reveals that notch... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=The%20SmoA1%20mouse%20model%20reveals%20that%20Notch%20signaling%20is%20critical%20for%20the%20growth%20and%20survival%20of%20sonic%20hedgehog-induced%20medulloblastomas. Accessed 29 Mar 2023

37. 'Park: Notch3 gene amplification in ovarian cancer - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Notch3%20gene%20amplification%20in%20ovarian%20cancer. Accessed 29 Mar 2023

38. Zhang H, Yang Y, Li X, Yuan X, Chu Q (2023) Targeting the Notch signaling pathway and the Notch ligand, DLL3, in small cell lung cancer. Biomed Pharmacother 159:114248. https://doi.org/10.1016/j.biopha.2023.114248

39. 'Expression analysis elucidates the roles of Nicastrin, Notch4, and Hes1 in prognosis and endocrine-therapy resistance in ER-positive breast cancer patients | Boustan | Research in Pharmaceutical Sciences'. http://rps.mui.ac.ir/index.php/jrps/article/view/2171/2227#. Accessed 23 Mar 2023

40. Luxán G, D'Amato G, MacGrogan D, de la Pompa JL (2016) Endocardial Notch signaling in cardiac development and disease. Circ Res 118(1):e1–e18. https://doi.org/10.1161/CIRCRESAHA.115.305350

41. Alberi L, Hoey SE, Brai E, Scotti AL, Marathe S (2013) Notch signaling in the brain: in good and bad times. Ageing Res Rev 12(3):801–814. https://doi.org/10.1016/j.arr.2013.03.004

42. Derry WB (2023) Role of developmental pathways in disease. FEBS J 290(13):3296–3299. https://doi.org/10.1111/febs.16873

43. Zhou B et al (2022) Notch signaling pathway: architecture, disease, and therapeutics. Signal Transduct Target Ther 7:95. https://doi.org/10.1038/s41392-022-00934-y

44. Kovall RA, Gebelein B, Sprinzak D, Kopan R (2017) The canonical Notch signaling pathway: structural and biochemical insights into shape, sugar, and force. Dev Cell 41(3):228–241. https://doi.org/10.1016/j.devcel.2017.04.001

45. Kopan R, Ilagan MXG (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 137(2):216–233. https://doi.org/10.1016/j.cell.2009.03.045

46. Fortini ME (2009) Notch signaling: the core pathway and its posttranslational regulation. Dev Cell 16(5):633–647. https://doi.org/10.1016/j.devcel.2009.03.010

47. Gordon WR, Arnett KL, Blacklow SC (2008) The molecular logic of Notch signalling: a structural and biochemical perspective. J Cell Sci 121(19):3109–3119. https://doi.org/10.1242/jcs.035683

48. Anusewicz D, Orzechowska M, Bednarek AK (2021) Notch signaling pathway in cancer: review with bioinformatic analysis. Cancers (Basel) 13(4):768. https://doi.org/10.3390/cancers13040768

49. Aster JC, Pear WS, Blacklow SC (2017) The varied roles of Notch in cancer. Annu Rev Pathol 12:245–275. https://doi.org/10.1146/annurev-pathol-052016-100127

50. 'Frontiers | Notch Signalling in Breast Development and Cancer'. https://www.frontiersin.org/articles/https://doi.org/10.3389/fcell.2021.692173/full. Accessed 14 Aug 2023

51. Yousefi H et al (2022) Notch signaling pathway: a comprehensive prognostic and gene expression profile analysis in breast cancer. BMC Cancer 22(1):1282. https://doi.org/10.1186/s12885-022-10383-z

52. Edwards A, Brennan K (2021) Notch signalling in breast development and cancer. Front Cell Dev Biol 9:692173. https://doi.org/10.3389/fcell.2021.692173

53. Han J, Hendzel MJ, Allalunis-Turner J (2011) Notch signaling as a therapeutic target for breast cancer treatment? Breast Cancer Res 13(3):210. https://doi.org/10.1186/bcr2875

54. Yuan X et al (2015) Expression of Notch1 correlates with breast cancer progression and prognosis. PLoS ONE 10(6):e0131689. https://doi.org/10.1371/journal.pone.0131689

55. Sui L et al (2020) Notch1 regulates breast cancer stem cell function via a non-canonical cleavage-independent pathway. biorxiv 26:1275. https://doi.org/10.1101/2020.02.28.970764

56. Gharaibeh L, Elmadany N, Alwosaibai K, Alshaer W (2020) Notch1 in cancer therapy: possible clinical implications and challenges. Mol Pharmacol 98(5):559–576. https://doi.org/10.1124/molpharm.120.000006

57. Capulli M et al (2019) Notch2 pathway mediates breast cancer cellular dormancy and mobilisation in bone and contributes to haematopoietic stem cell mimicry. Br J Cancer 121(2):157–171. https://doi.org/10.1038/s41416-019-0501-y

58. Pancewicz J (2020) A brief overview of clinical significance of novel Notch2 regulators. Mol Cellul Oncol 7(5):1776084. https://doi.org/10.1080/23723556.2020.1776084

59. Aburjania Z, Jang S, Whitt J, Jaskula-Stzul R, Chen H, Rose JB (2018) The role of Notch3 in cancer. Oncologist 23(8):900–911. https://doi.org/10.1634/theoncologist.2017-0677

60. Zhang Y-Q et al (2021) Notch3 inhibits cell proliferation and tumorigenesis and predicts better prognosis in breast cancer through transactivating PTEN. Cell Death Dis. https://doi.org/10.1038/s41419-021-03735-3

61. Xiu M et al (2021) The role of Notch3 signaling in cancer stemness and chemoresistance: molecular mechanisms and targeting strategies. Front Mol Biosci. https://doi.org/10.3389/fmolb.2021.694141

62. Landor SKJ et al (2021) PIM-induced phosphorylation of Notch3 promotes breast cancer tumorigenicity in a CSL-independent fashion. J Biol Chem 296:100593. https://doi.org/10.1016/j.jbc.2021.100593

63. Tian Y et al (2023) Silencing Notch4 promotes tumorigenesis and inhibits metastasis of triple-negative breast cancer via Nanog and Cdc42. Cell Death Discov. https://doi.org/10.1038/s41420-023-01450-w

64. Xiu M, Zeng X, Shan R, Wen W, Li J, Wan R (2021) Targeting Notch4 in cancer: molecular mechanisms and therapeutic

perspectives. Cancer Manag Res 13:7033–7045. https://doi.org/10.2147/CMAR.S315511

65. Rauff B, Malik A, Bhatti YA, Chudhary SA, Qadri I, Rafiq S (2020) Notch signalling pathway in development of cholangiocarcinoma. World J Gastrointest Oncol 12(9):957–974. https://doi.org/10.4251/wjgo.v12.i9.957

66. 'Chyla: Genetic biomarkers of sensitivity and resistance... - Google Scholar'. https://scholar.google.com/scholar_lookup?&title=Genetic%20biomarkers%20of%20sensitivity%20and%20resistance%20to%20venetoclax%20monotherapy%20in%20patients%20with%20relapsed%20acute%20myeloid%20leukemia&journal=American%20Journal%20of%20Hematology&volume=93&publication_year=2018&author=Chyla%2CB&author=Daver%2CN&author=Doyle%2CK&author=Mckeegan%2CE&author=Huang%2CX&author=Ruvolo%2CV&author=Wang%2CZ&author=Chen%2CK&author=Souers%2CA&author=Leverson%2CJ. Accessed 29 Mar 2023

67. 'Banerjee: CDK4 in lung, and head and neck cancers... - Google Scholar'. https://scholar.google.com/scholar_lookup?&title=CDK4%20in%20lung%2C%20and%20head%20and%20neck%20cancers%20in%20old%20age%3A%20Evaluation%20as%20a%20biomarker&journal=Clinical%20and%20Translational%20Oncology&volume=19&pages=571-578&publication_year=2017&author=Banerjee%2CJ&author=Pradhan%2CR&author=Gupta%2CA&author=Kumar%2CR&author=Sahu%2CV&author=Upadhyay%2CD&author=Chaterjee%2CP&author=Dwivedi%2CS&author=Dey%2CS&author=Dey%2CAB. Accessed 29 Mar 2023

68. Starzer AM, Berghoff AS, Bartsch R (2023) Biomarkers and translational research approaches in breast cancer: an update. Memo 16(1):42–46. https://doi.org/10.1007/s12254-022-00855-0

69. Ogier du Terrail J et al (2023) Federated learning for predicting histological response to neoadjuvant chemotherapy in triple-negative breast cancer. Nat Med 29(1):135. https://doi.org/10.1038/s41591-022-02155-w

70. Mehmood A, Nawab S, Jin Y, Hassan H, Kaushik AC, Wei D-Q (2023) Ranking breast cancer drugs and biomarkers identification using machine learning and pharmacogenomics. ACS Pharmacol Transl Sci 6(3):399–409. https://doi.org/10.1021/acsptsci.2c00212

71. Pilvenyte G, Ratautaite V, Boguzaite R, Ramanavicius A, Viter R, Ramanavicius S (2023) Molecularly imprinted polymers for the determination of cancer biomarkers. Int J Mol Sci 24(4):4105. https://doi.org/10.3390/ijms24044105

72. 'Notch signalling in solid tumours: a little bit of everything but not all the time | Nature Reviews Cancer'. https://www.nature.com/articles/nrc3035. Accessed 26 May 2023

73. Bolós V, Blanco M, Medina V, Aparicio G, Díaz-Prado S, Grande E (2009) Notch signalling in cancer stem cells. Clin Transl Oncol 11(1):11–19. https://doi.org/10.1007/s12094-009-0305-2

74. Krishna BM et al (2019) Notch signaling in breast cancer: from pathway analysis to therapy. Cancer Lett 461:123–131. https://doi.org/10.1016/j.canlet.2019.07.012

75. 'Molecular pathways: translational and therapeutic implications of the notch signaling pathway in cancer| Clinical Cancer Research| American Association for Cancer Research'. https://aacrjournals.org/clincancerres/article/21/5/955/247574/Molecular-Pathways-Translational-and-Therapeutic. Accessed 26 May 2023

76. 'Luteolin inhibits breast cancer development and progression in vitro and in vivo by suppressing notch signaling and regulating MiRNAs| Cellular Physiology and Biochemistry| Karger Publishers'. https://karger.com/cpb/article/37/5/1693/73537/Luteolin-Inhibits-Breast-Cancer-Development-and. Accessed 26 May 2023

77. Duffy MJ, Synnott NC, Crown J (2018) Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. Breast Cancer Res Treat 170(2):213–219. https://doi.org/10.1007/s10549-018-4753-7

78. Ma H et al (2010) Use of four biomarkers to evaluate the risk of breast cancer subtypes in the women's contraceptive and reproductive experiences study. Can Res 70(2):575–587. https://doi.org/10.1158/0008-5472.CAN-09-3460

79. Beenken SW et al (2001) Molecular biomarkers for breast cancer prognosis: coexpression of c-erbB-2 and p53. Ann Surg 233(5):630

80. 'Mutant p53 in breast cancer: potential as a therapeutic target and biomarker | SpringerLink'. https://doi.org/10.1007/s10549-018-4753-7 . Accessed 29 Mar 2023

81. Synnott NC et al (2017) Mutant p53: a novel target for the treatment of patients with triple-negative breast cancer? Int J Cancer 140(1):234–246. https://doi.org/10.1002/ijc.30425

82. 'Echle: Deep learning in cancer pathology: a new generatio... - Google Scholar'. https://scholar.google.com/scholar_lookup?&title=Deep%20learning%20in%20cancer%20pathology%3A%20A%20new%20generation%20of%20clinical%20biomarkers&journal=British%20Journal%20of%20Cancer&volume=124&pages=686-696&publication_year=2021&author=Echle%2CA&author=Rindtorff%2CNT&author=Brinker%2CTJ&author=Luedde%2CT&author=Pearson%2CAT&author=Kather%2CJN. Accessed 29 Mar 2023

83. Marima R et al (2023) Application of AI in novel biomarkers detection that induces drug resistance, enhance treatment regimens, and advancing precision oncology. In: Dlamini Z (ed) artificial intelligence and precision oncology: bridging cancer research and clinical decision support. Springer Nature Switzerland, Cham, pp 29–48

84. Filippini T et al (2020) Green tea (*Camellia sinensis*) for the prevention of cancer. Cochrane Database Syst Rev 2020(3):CD005004. https://doi.org/10.1002/14651858.CD005004.pub3

85. Yang CS, Wang X, Lu G, Picinich SC (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9(6):429. https://doi.org/10.1038/nrc2641

86. Li M-J, Yin Y-C, Wang J, Jiang Y-F (2014) Green tea compounds in breast cancer prevention and treatment. World J Clin Oncol 5(3):520–528. https://doi.org/10.5306/wjco.v5.i3.520

87. Iwasaki M et al (2010) Green tea drinking and subsequent risk of breast cancer in a population to based cohort of Japanese women. Breast Cancer Res 12(5):R88. https://doi.org/10.1186/bcr2756

88. Rather RA, Bhagat M (2018) Cancer chemoprevention and piperine: molecular mechanisms and therapeutic opportunities. Front Cell Dev Biol 6:10. https://doi.org/10.3389/fcell.2018.00010

89. Mitra S et al (2022) Anticancer applications and pharmacological properties of piperidine and piperine: a comprehensive review on molecular mechanisms and therapeutic perspectives. Front Pharmacol. https://doi.org/10.3389/fphar.2021.772418

90. Habib SHM, Makpol S, Hamid NAA, Das S, Ngah WZW, Yusof YAM (2008) Ginger extract (*Zingiber officinale*) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics 63(6):807–813. https://doi.org/10.1590/S1807-59322008000600017

91. Mahomoodally MF et al (2021) Ginger and its active compounds in cancer therapy: from folk uses to nano-therapeutic applications. Semin Cancer Biol 69:140–149. https://doi.org/10.1016/j.semcancer.2019.08.009

92. Promdam N, Panichayupakaranant P (2022) [6]-Gingerol: a narrative review of its beneficial effect on human health. Food Chem Adv 1:100043. https://doi.org/10.1016/j.focha.2022.100043

93. Khan A, Chen H, Tania M, Zhang D (2011) Anticancer activities of nigella sativa (black cumin). Afr J Tradit Complement Altern Med 8(5 Suppl):226–232. https://doi.org/10.4314/ajtcam.v8i5S.10

94. Oh J-W, Muthu M, Pushparaj SSC, Gopal J (2023) Anticancer therapeutic effects of green tea catechins (GTCs) when integrated with antioxidant natural components. Molecules 28(5):2151. https://doi.org/10.3390/molecules28052151

95. LaVoie MJ, Selkoe DJ (2003) The Notch ligands, Jagged and Delta, are sequentially processed by alpha-secretase and presenilin/gamma-secretase and release signaling fragments. J Biol Chem 278(36):34427–34437. https://doi.org/10.1074/jbc.M30265 9200

96. Lakshmi SP, Reddy AT, Kodidhela LD, Varadacharyulu NC (2020) The tea catechin epigallocatechin gallate inhibits NF-κB-mediated transcriptional activation by covalent modification. Arch Biochem Biophys 695:108620. https://doi.org/10.1016/j.abb.2020.108620

97. Varghese E, Samuel SM, Abotaleb M, Cheema S, Mamtani R, Büsselberg D (2018) The "Yin and Yang" of natural compounds in anticancer therapy of triple-negative breast cancers. Cancers 10(10):346. https://doi.org/10.3390/cancers10100346

98. Lechner JF, Stoner GD (2019) Gingers and their purified components as cancer chemopreventative agents. Molecules 24(16):2859. https://doi.org/10.3390/molecules24162859

99. '(10) Gingerol - an overview | ScienceDirect Topics'. https://www.sciencedirect.com/topics/medicine-and-dentistry/10-gingerol. Accessed 15 Aug 2023

100. Ke X et al (2015) TQ inhibits hepatocellular carcinoma growth in vitro and in vivo via repression of Notch signaling. Oncotarget 6(32):32610–32621

101. Adinew GM, Messeha SS, Taka E, Badisa RB, Antonie LM, Soliman KFA (2022) Thymoquinone alterations of the apoptotic gene expressions and cell cycle arrest in genetically distinct triple-negative breast cancer cells. Nutrients 14(10):2120. https://doi.org/10.3390/nu14102120

102. Liu Y, Yang X, Gan J, Chen S, Xiao Z-X, Cao Y (2022) CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Res 50(W1):W159–W164. https://doi.org/10.1093/nar/gkac394

103. Driggins SN (2005) The anti-carcinogenic effect of *Echinacea purpurea* and *Echinacea pallida* on a mammalian breast cancer cell line. PhD Thesis, Tennessee State University

104. 'Galeone: Onion and garlic use and human cancer - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Onion%20and%20garlic%20use%20and%20human%20cancer&publication_year=2006&author=C.%20Galeone&author=C.%20Pelucchi&author=F.%20Levi&author=E.%20Negri&author=S.%20Franceschi&author=R.%20Talamini&author=A.%20Giacosa&author=C.%20La%20Vecchia. Accessed 29 Mar 2023

105. 'Craig: Health-promoting properties of common herbs - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Health-promoting%20properties%20of%20common%20herbs&publication_year=1999&author=J.C.%20Winston. Accessed 29 Mar 2023

106. Tamayo C, Richardson MA, Diamond S, Skoda I (2000) The chemistry and biological activity of herbs used in Flor-Essence™ herbal tonic and Essiac. Phytother Res 14(1):1–14

107. 'Zaveri: Green tea and its polyphenolic catechins:... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Green%20tea%20and%20its%20polyphenolic%20catechins%3A%20medicinal%20uses%20in%20cancer%20and%20noncancerous%20applications&publication_year=2006&author=N.T.%20Zaveri. Accessed 29 Mar 2023

108. Shareef M, Ashraf MA, Sarfraz M (2016) Natural cures for breast cancer treatment. Saudi Pharm J 24(3):233–240. https://doi.org/10.1016/j.jsps.2016.04.018

109. 'Rockwell: Alteration of the effects of cancer therapy... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Alteration%20of%20the%20effects%20of%20cancer%20therapy%20agents%20on%20breast%20cancer%20cells%20by%20the%20herbal%20medicine%20black%20cohosh&publication_year=2005&author=S.%20Rockwell&author=Y.%20Liu&author=S.A.%20Higgins. Accessed 29 Mar 2023

110. 'Tan: Mammary gland morphogenesis is enhanced by exposure... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Mammary%20gland%20morphogenesis%20is%20enhanced%20by%20exposure%20to%20flaxseed%20or%20its%20major%20lignan%20during%20suckling%20in%20rats&publication_year=2004&author=K.P.%20Tan&author=J.%20Chen&author=W.E.%20Ward&author=L.U.%20Thompson. Accessed 29 Mar 2023

111. 'Elkady: Differential control of growth, apoptotic... - Google Scholar'. https://scholar.google.com/scholar_lookup?title=Differential%20control%20of%20growth%2C%20apoptotic%20activity%2C%20and%20gene%20expression%20in%20human%20breast%20cancer%20cells%20by%20extracts%20derived%20from%20medicinal%20herbs%20Zingiber%20officinale&publication_year=2012&author=A.I.%20Elkady&author=O.A.%20Abuzinadah&author=N.A.%20Baeshen&author=T.R.%20Rahmy. Accessed 29 Mar 2023

112. Takooree H et al (2019) A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications. Crit Rev Food Sci Nutr 59(sup1):S210–S243. https://doi.org/10.1080/10408398.2019.1565489

113. Mahmoud SS, Torchilin VP (2013) Hormetic/cytotoxic effects of nigella sativa seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. Cell Biochem Biophys 66(3):451–460. https://doi.org/10.1007/s12013-012-9493-4

114. Jumper J et al (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596(7873):583–589. https://doi.org/10.1038/s41586-021-03819-2

115. 'Chopra: Ayurvedic medicine: core concept, therapeutic... - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=Med+Clin+North+Am&title=Ayurvedic+medicine.+Core+concept,+therapeutic+principles,+and+current+relevance&volume=86&issue=1&publication_year=2002&pages=75-89,+vii&pmid=11795092&doi=10.1016/S0025-7125(03)00073-7. Accessed 26 May 2023

116. 'Conboy: Ayurveda and Panchakarma: measuring the effects... - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=ScientificWorldJournal&title=Ayurveda+and+Panchakarma:+measuring+the+effects+of+a+holistic+health+intervention&volume=9&publication_year=2009&pages=272-280&pmid=19412555&doi=10.1100/tsw.2009.35. Accessed 26 May 2023

117. Koul B (2019) Herbs for cancer treatment. Springer, Singapore

118. 'Rao: Opportunities and challenges in Ayurveda: global... - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=Altern+Integr+Med&title=Opportunities+and+challenges+in+Ayurveda:+global+perspective&volume=6&issue=2&publication_year=2017&pages=239. Accessed 26 May 2023

119. Khoobchandani M et al (2020) New approaches in breast cancer therapy through green nanotechnology and nano-ayurvedic medicine: pre-clinical and pilot human clinical investigations. Int J Nanomed 15:181–197. https://doi.org/10.2147/IJN.S219042

120. 'Buckner: Complementary and alternative medicine use... - Google Scholar'. https://scholar.google.com/scholar_lookup?

journal=Curr+Oncol&title=Complementary+and+alternative+medicine+use+in+patients+before+and+after+a+cancer+diagnosis&volume=25&issue=4&publication_year=2018&doi=10.3747/co.25.3884. Accessed 26 May 2023

121. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A (2023) IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS Omega 8(9):8827–8845. https://doi.org/10.1021/acsomega.3c00156

122. 'ChEMBL Database'. https://www.ebi.ac.uk/chembl/. Accessed 14 Apr 2023

123. 'admetSAR'. http://lmmd.ecust.edu.cn/admetsar2. Accessed 14 Apr 2023

124. 'CB-Dock: a web server for cavity detection-guided protein–ligand blind docking. Acta Pharm Sin'. https://www.nature.com/articles/s41401-019-0228-6. Accessed 16 June 2023

125. Ferdous S, Mirza U, Saeed U (2013) Docking studies reveal phytochemicals as the long searched anticancer drugs for breast cancer. Int J Comput Appl. https://doi.org/10.5120/11740-7073

126. Deng F, Ghemtio L, Grazhdankin E, Wipf P, Xhaard H, Kidron H (2020) Binding site interactions of modulators of breast cancer resistance protein, multidrug resistance-associated protein 2, and P-glycoprotein activity. Mol Pharm 17(7):2398–2410. https://doi.org/10.1021/acs.molpharmaceut.0c00155

127. 'CB-Dock: An accurate protein-ligand blind docking tool'. http://clab.labshare.cn/cb-dock/php/index.php. Accessed 14 Apr 2023

128. Stank A, Kokh DB, Fuller JC, Wade RC (2016) Protein binding pocket dynamics. Acc Chem Res 49(5):809–815. https://doi.org/10.1021/acs.accounts.5b00516

129. Santos R, Ferreira L, Andricopulo A (2018) Practices in molecular docking and structure-based virtual screening. Springer, New York

130. 'Molecular Dynamics Simulation'. https://www.cgl.ucsf.edu/chimera/docs/ContributedSoftware/md/md.html. Accessed 14 Apr 2023

131. Guan L et al (2018) ADMET-score: a comprehensive scoring function for evaluation of chemical drug-likeness. Medchemcomm 10(1):148–157. https://doi.org/10.1039/c8md00472b

132. Abdul-Hammed M, Adedotun IO, Falade VA, Adepoju AJ, Olasupo SB, Akinboade MW (2021) Target-based drug discovery, ADMET profiling and bioactivity studies of antibiotics as potential inhibitors of SARS-CoV-2 main protease (Mpro). Virus Dis 32(4):642–656. https://doi.org/10.1007/s13337-021-00717-z

133. 'Hinkson: Accelerating therapeutics for opportunities... - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=Front+Pharmacol&title=Accelerating+therapeutics+for+opportunities+in+medicine:+a+paradigm+shift+in+drug+discovery&author=I.V.+Hinkson&author=B.+Madej&author=E.A.+Stahlberg&volume=11&publication_year=2020&pages=770&pmid=32694991. Accessed 1 June 2023

134. 'Dowden: Trends in clinical success rates and therapeutic... - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=Nat+Rev+Drug+Discov&title=Trends+in+clinical+success+rates+and+therapeutic+focus&author=H.+Dowden&author=J.+Munro&volume=18&publication_year=2019pages=495-496. Accessed 1 June 2023

135. 'Harrison: Phase II and phase III failures: 2013–2015 - Google Scholar'. https://scholar.google.com/scholar_lookup?journal=Nat+Rev+Drug+Discov&title=Phase+II+and+phase+III+failures:+2013%E2%80%922015&author=R.K.+Harrison&volume=15&publication_year=2016&pages=817-818&pmid=27811931. Accessed 1 June 2023

136. Wang J (2009) Comprehensive assessment of ADMET risks in drug discovery. Curr Pharm Des 15(19):2195–2219. https://doi.org/10.2174/138161209788682514

137. Sliwoski G, Kothiwale S, Meiler J, Lowe EW (2014) Computational methods in drug discovery. Pharmacol Rev 66(1):334–395. https://doi.org/10.1124/pr.112.007336

138. Fu Y, Zhao J, Chen Z (2018) Insights into the molecular mechanisms of protein-ligand interactions by molecular docking and molecular dynamics simulation: a case of oligopeptide binding protein. Comput Math Methods Med 2018:3502514. https://doi.org/10.1155/2018/3502514

139. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31(2):455–461. https://doi.org/10.1002/jcc.21334

140. Xue Q et al (2022) Evaluation of the binding performance of flavonoids to estrogen receptor alpha by autodock, autodock vina and surflex-dock. Ecotoxicol Environ Saf 233:113323. https://doi.org/10.1016/j.ecoenv.2022.113323

141. Meng X-Y, Zhang H-X, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7(2):146–157

142. Shaikh SA, Jain T, Sandhu G, Latha N, Jayaram B (2007) From drug target to leads–sketching a physicochemical pathway for lead molecule design in silico. Curr Pharm Des 13(34):3454–3470. https://doi.org/10.2174/138161207782794220

143. Kumar V (2020) RETRACTED ARTICLE: Seven spices of India—from kitchen to clinic. J Ethn Foods 7(1):23. https://doi.org/10.1186/s42779-020-00058-0

144. Bozorgi A, Khazaei S, Khademi A, Khazaei M (2020) Natural and herbal compounds targeting breast cancer, a review based on cancer stem cells. Iran J Basic Med Sci 23(8):970–983. https://doi.org/10.22038/ijbms.2020.43745.10270