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# IN SILICO EVALUATION OF PLANT-DERIVED BIOACTIVE COMPOUNDS TARGETING THE mTOR PATHWAY IN BREAST CANCER: PICROSIDE II AS A PROMISING NATURAL INHIBITOR

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## Abstract

The PI3K/AKT/mTOR signaling pathway plays a central role in breast cancer development, as it governs crucial cellular activities such as growth, proliferation, and survival. This pathway is frequently disrupted in hormone receptor-positive breast cancers. Though mTOR inhibitors, such as rapamycin and its analog (rapalog), have shown promise in treatment, their clinical use is often hindered by undesirable side effects. Therefore, the development of novel inhibitors targeting the mTOR pathway is crucial. This study investigates the binding efficiency and molecular interactions of several anti-carcinogenic bioactive compounds namely Vindoline, Vincristine, Vinblastine, Picroside, and And Cucurbitacin B against the mTOR complex using the Schrödinger Suite for molecular docking analysis. These compounds, derived from medicinal plants traditionally used in cancer therapies, including those in Siddha and other traditional medicine systems, have demonstrated therapeutic promise. Among the evaluated compounds, Picroside II, isolated from Picrorhiza kurroa, a medicinal herb extensively used in traditional Chinese medicine, showed the highest binding affinity (-12.134 kcal/mol) for mTOR, surpassing that of the reference inhibitor rapamycin. These results indicate that Picroside II could be a promising lead compound for further research in breast cancer therapy, with the potential to offer benefits over current mTOR inhibitors. These results indicate that Picroside II could be a promising lead compound for further research in the treatment of cancer, with the potential to provide improved efficacy compared to existing mTOR inhibitors.

***Keywords***: mTOR Inhibition, Breast Cancer, Plant-Derived Bioactive Compounds, Picroside II, Molecular Docking, Schrödinger Suite.

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## INTRODUCTION

Breast cancer is a heterogeneous disease with several subtypes, typically classified based on the status of the hormone receptor and the human epidermal growth factor receptor 2 (HER2) expression [1, 2]. Luminal A is the most common breast cancer subtype, representing almost 50–60% of all cases [3]. It is characterized by the presence of estrogen and/or progesterone receptors and the absence of HER2 expression [4].

The PI3K/AKT/mTOR (PAM) pathway is a conserved eukaryotic signaling cascade that regulates cell growth, survival, and proliferation. Its dysregulation, often through crosstalk with other pathways, contributes to oncogenesis [5]. Around one-quarter of breast cancer cases show abnormalities in this pathway, which is strongly associated with resistance to endocrine therapy [6]. This resistance is partly due to AKT's ability to activate estrogen receptor (ER) signaling independently of estrogen [7]. Therefore, mTOR inhibitors are often used in combination with endocrine therapy to improve therapeutic outcomes [8, 9].

The mechanistic target of rapamycin (mTOR), also known as FRAP1, is a serine/threonine kinase belonging to the phosphoinositide 3-kinase-related kinase (PIKK) family [10]. It is a large protein consisting of 2,549 amino acids (~289 kDa), comprising five distinct functional domains: i) HEAT (Huntington, elongation factor 3, PR65 /A, TOR), ii) FAT (FRAP, ATM, TRRAP), iii) FRB (the binding site for FKBP12-rapamycin complex), iv) the kinase domain, and v) the FATC domain (FRAP, ATM, TRRAP carboxy terminus) [11]. mTOR functions as a catalytic subunit in two distinct complexes: mTORC1 and mTORC2 [12]. While both share catalytic subunits, they differ in specific accessory proteins: raptor and rictor in mTORC1 and mTORC2 respectively. mTORC1 is responsive to nutrient signals and regulates various metabolic processes, whereas mTORC2 mainly controls cytoskeletal organization and is nutrient-insensitive [13].

There are three major strategies to inhibit mTOR: (i) ATP-competitive inhibitors targeting the kinase domain [14]; (ii) inhibitors targeting DFG-out conformation; and (iii) allosteric inhibitors, such as rapamycin, which bind to the FRB domain. While ATP-competitive inhibitors affect both mTORC1 and mTORC2 and induce apoptosis, they often lack specificity due to the conserved nature of ATP-binding sites and high intracellular ATP concentrations. DFG-out targeting is challenging due to limited structural data. Allosteric inhibitors like rapamycin, and its analogs (rapalogs) such as everolimus [16], bind the FKBP12-rapamycin complex to the FRB domain [17], blocking kinase activity of mTORC1 [15,18]. Though effective, mTORC2 remains largely resistant to these drugs, although prolonged exposure may eventually inhibit it as well.

Despite therapeutic benefits, rapamycin and rapalogs are associated with adverse effects due to their immunosuppressive properties, including stomatitis [19], hyperglycemia [20], fatigue, hyperlipidemia [21] and non-infectious pneumonitis [22]. They also activate feedback loops that upregulate PI3K/AKT signaling, potentially enhancing cancer cell survival [23]. Therefore, identifying novel compounds with better pharmacological profiles is crucial for the effective treatment of ER-positive breast cancer.

Medicinal plants have long been explored for their anti-cancer properties [24], and bioactive compounds from traditional Chinese medicine (TCM) have shown promise as potential anti-cancer agents [25]. This study investigates the potential of selected plant-derived bioactive compounds-Picroside II, Cucurbitacin B, Vindoline, Vincristine, and Vinblastine-as mTOR inhibitors for the treatment of endocrine receptor-positive breast cancer. Given the limitations and adverse effects associated with existing mTOR inhibitors, this study aims to identify alternative natural compounds with improved pharmacological profiles and fewer side effects. Using molecular docking and computational analysis via the Schrödinger Suite, the binding efficiency and molecular interactions of these compounds with the mTOR complex, particularly targeting the FRB domain, was assessed. The findings contribute to the ongoing search for safer and more effective plant-based mTOR inhibitors to overcome the resistance in breast cancer treatment.

## MATERIALS AND METHODS

### Protein Preparation

The crystal structure of human FKBP12, rapamycin, and the FKBP12-rapamycin-binding (FRB) domain of the Human FRAP/mTOR protein (PDB ID -1FAP) was retrieved from the Protein Data Bank (PDB) at a 2.7 Å resolution. To ensure structural accuracy for docking simulations, the protein was pre-processed using the Protein Preparation Wizard tool available in Maestro V9.3.5 (Schrödinger Suite). The preprocessing involved addition of hydrogen atoms, assignment of bond orders, correction of bond lengths, and removal of crystallographic water molecules beyond 5 Å from the binding site. Protonation states of residues were optimized at pH 7.0. Finally, the structure was then energy-minimized using the OPLS 2005 force field with default parameters to eliminate steric clashes and reduce geometric strain

### Ligand Preparation

The 3D structures of the five selected bioactive phytochemicals: Picroside II (PubChem CID: 9849283), Cucurbitacin B (CID: 5281316), Vinblastine (CID: 13342), Vindoline (CID: 260535), and Vincristine (CID: 5978) were retrieved from the NCBI PubChem Compound Database in SD Format. These ligands were processed using the LigPrep module in Schrödinger Suite 2012, which optimizes geometry and generates low-energy 3D conformers suitable for molecular docking. Table 1 summarizes the chemical structures and the key physicochemical properties of the selected compounds, while their 3D conformations are depicted in Figure 1.

Table 1 List of the selected bioactive compounds along with their corresponding PubChem Compound IDs

| S. No | Compound Name | PubChem ID |
|---|---|---|
| 1 | Picroside II | 9849283 |
| 2 | Cucurbitacin B | 5281316 |
| 3 | Vinblastine | 13342 |
| 4 | Vindoline | 260535 |
| 5 | Vincristine | 5978 |

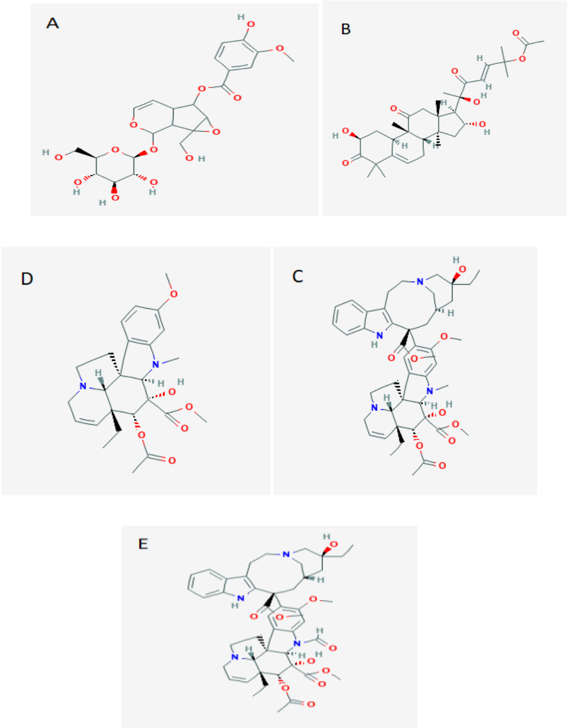

Figure 1: Chemical structures of bio-active compounds selected for this study. (A) Picroside II (B) Cucurbitacin B (C) Vinblastine (D) Vindoline (E) Vincristine.

### Receptor Grid Generation

A receptor grid was constructed centered on the native ligand rapamycin, which remained in the processed protein structure to define the active binding pocket for molecular docking. The grid generation step in Schrödinger allows precise mapping of the binding site and defines the spatial coordinates where ligands will be evaluated during docking. This ensures that the docked ligands are assessed in a biologically relevant pocket, maximizing docking accuracy.

### Molecular Docking

The ligands were docked into the generated receptor grid using the Glide XP module (extra-precision) in the Schrödinger Suite. This extra precision algorithm evaluates binding affinity based on hydrophobic, hydrogen bonding, electrostatic, and desolvation interactions. The software generated multiple binding poses for each compound and ranked them based on Glide Score. The lowest energy pose for each compound was selected for further analysis to evaluate binding efficiency and molecular interactions with the FRB domain of mTOR.

## RESULTS

### Molecular Docking Studies and Ligand Binding Affinities

Molecular docking was conducted using Schrödinger Maestro V9.3, a robust computational platform, to investigate the interaction profiles and binding affinities of selected plant-derived bioactive compounds with the FRB domain of the mTOR protein. The potential inhibitors were identified based on their low docking scores indicating their stronger binding affinity and favorable binding energies for stable the ligand-protein complex. The docking results, including scoring parameters and specific molecular interactions, are presented in Table 2 and presented in Figure 02.

Table 02 Molecular docking results of selected bioactive compounds against FKBP12 domain of mTOR

| S. No | Compound Name | Docking Score (kcal/mol) | Glide Score (kcal/mol) | XP Score (kcal/mol) | Glide Energy (kcal/mol) | Hydrogen bond interaction |
|---|---|---|---|---|---|---|
| 1 | Picroside II | -12.134 | -12.134 | -12.134 | -61.595 | Glu52, Ile56 |
| 2 | Cucurbitacin B | -10.426 | -10.426 | -10.426 | -47.639 | Tyr26, Arg42, Ile56 |
| 3 | Vinblastine | -7.384 | -8.918 | -8.918 | -42.695 | Arg42, Asp37 |
| 4 | Vindoline | -6.715 | -6.771 | -6.771 | -47.253 | Arg42, Asp37 |
| 5 | Vincristine | -6.287 | -6.473 | -6.473 | -6.325 | Tyr82, Glu 54 |

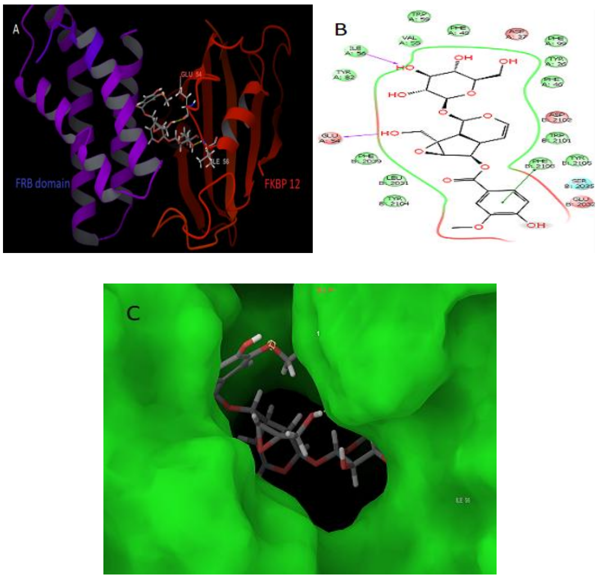

Figure 2: (A) Docking pose of Picroside II (B) Interaction of Picroside II with mTOR (C) Molecular representation of mTOR complexed with Picroside II.

### Compound A: Picroside II (PubChem ID: 9849283)

Among the five compounds tested, Picroside II showed the highest binding affinity, achieving a docking score of –12.134 kcal/mol and a binding energy of -61.595 kcal/mol. This compound established hydrogen bonds with Glu52 and Ile56 within the FKBP12-rapamycin binding interface. Additionally, π–π stacking interactions with Phe2106 in the FRB domain of mTOR contributed to a stable binding conformation, suggesting its high potential as a strong mTOR inhibitor.

### Compound B: Cucurbitacin B (PubChem ID: 5281316)

Cucurbitacin B demonstrated the second strongest binding affinity, with a docking score of –10.426 kcal/mol and a binding energy of -47.639 kcal/mol. It formed three hydrogen bonds with Tyr26, Arg42, and Ile56 at the FKBP12-rapamycin binding site. These strong interactions highlight its potential as an effective modulator of the mTOR signaling pathway.

### Compound C: Vinblastine (PubChem ID: 13342)

Vinblastine exhibited a moderate binding affinity, showing a docking score of -7.384 kcal/mol and a binding energy of -42.695 kcal/mol. It formed hydrogen bonds with the Arg42 and Asp37 residues and engaged in π–π interactions with Phe2039 within the FRB domain, supporting a stable but less potent interaction compared to compounds A and B.

### Compound D: Vindoline (PubChem ID: 260535)

Vindoline demonstrated a comparable binding affinity to vinblastine, with a docking score of –6.715 kcal/mol and a binding energy of -47.253 kcal/mol. It formed hydrogen bonds with the residues Arg42 and Asp37 along with π–π stacking interactions with Phe2039. Despite a slightly lower docking score, its favorable binding energy suggests a thermodynamically stable complex.

### Compound E: Vincristine (PubChem ID: 5978)

Vincristine exhibited the lowest binding affinity among the tested compounds, with a docking score of -6.287 kcal/mol and a binding energy of -6.325 kcal/mol. The hydrogen bonds were formed with Tyr82 and Glu54 within the FKBP12-rapamycin interface. The relatively low interaction strength indicates limited potential for effective inhibition of the mTOR FRB domain.

## DISCUSSION

The molecular docking analysis results highlighted the efficacy of various phytochemical compounds including Cucurbitacin B, Picroside, Vinblastine, Vindoline, And Vincristine - against mTOR in breast cancer. Among all the compounds studied Picroside [II], a compound derived from *Picrorhiza kurroa*, demonstrated greater efficacy than the aforementioned commercial drug targets. Picroside II exhibited the highest affinity for FKBP [12] among all the phytochemicals analyzed. Given its safety for oral administration, Picroside II could serve as a promising candidate for the development of breast cancer therapy.

Approximately one in every eight women is at risk of developing breast cancer at some stage in her life [26]. Although recent advancements in cytotoxic chemotherapy and targeted therapies have improved survival rates, breast cancer still causes over 40,000 deaths annually in the United States, according to the American Cancer Society's 2017 report [27]. Key risk factors for breast cancer include age [28], family history [29], alcohol consumption [30], obesity [31], and physical inactivity 32. Breast cancer can be hereditary or sporadic. Hereditary factors account for 5%–10% of breast cancer cases, primarily due to germline mutations in the BRCA1 and BRCA2 genes33. In contrast, sporadic breast cancer, accounting for approximately 85% of cases, is often associated with alterations in MYC, CCND1 (Cyclin D1), and ERBB2 (HER2/neu) genes [34].

Breast cancer is a complex and heterogeneous disease. About 75% of breast cancers are ER and/or PR positive, with premenopausal women comprising at least half of these cases [20]. The luminal A subtype is the most prevalent, representing 50–60% of all breast cancer cases [35]. Sub types are categorized according to the expression status of the HER2 receptor, progesterone receptor (PR), and estrogen receptor (ER) [36]. Accordingly, breast cancers are categorized into three major subtypes: triple-negative or basal-like (TNBC), HER2-positive, and luminal (ER/PR-positive). Luminal subtypes are further divided into luminal A (ER/PR+, HER2–, low Ki67 expression) and luminal B (ER/PR+, HER2+, high Ki67 expression). HER2-positive tumors are ER/PR–, while triple-negative tumors lack all three receptors [37].

The Cancer Genome Atlas Network identified key genetic mutations in breast cancer subtypes. Luminal/ER-positive tumors, particularly luminal A, show a high frequency of PIK3CA mutations, also common in HER2-positive tumors. Although less frequent, PTEN loss remains significant. Mutations in PIK3CA, PTEN, and AKT1 are generally mutually exclusive, though co-mutations of PIK3CA and PTEN occur in other cancers [38]. Recent studies show that the PI3K pathway can be activated independently of Akt, and PIK3CA mutations may cause tumorigenesis through Akt-independent ways [39,40].

The PI3K /Akt /mTOR pathway plays a vital role in cellular metabolism and regulates various events like cell growth, cell proliferation, cell survival, adhesion, motility and differentiation [6]. Dysregulation of this pathway is associated with various cancer hallmarks, including uncontrolled cell proliferation, genomic instability, and metabolic reprogramming [41,42]. The loss of PTEN function, a tumor suppressor gene, leads to mTOR hyperactivation which in turn promotes carcinogenesis through increased expression of mRNAs that code for growth factors, angiogenic factors, and inhibitors of apoptosis [43]. As a result, mTOR has become a key therapeutic target in the treatment of cancer.

Given the frequent dysregulation of the PI3K/Akt/mTOR pathway in breast cancer and its critical role in promoting tumor growth and survival, therapeutic strategies have increasingly focused on targeting this signaling cascade. One such approach involves the use of mTOR inhibitors like rapamycin and its analogs. Rapamycin, a potent immunosuppressive agent isolated from *Streptomyces hygroscopicus*, binds to FKBP12 and FRAP protein. Rapamycin's structure allows it to occupy two distinct hydrophobic binding pockets, enabling strong interactions with both proteins, though the inter-protein interactions are less robust [44]. Due to issues with solubility and stability, analogs of rapamycin, known as rapalogs, have been developed. Everolimus an mTOR inhibitor currently approved by the FDA for breast cancer therapy [45]. While its side effects are generally manageable, it has been associated with adverse events in clinical trials. This study aimed to identify phytochemicals from medicinal plants that could serve as effective alternatives with fewer side effects.

Historically, medicinal plants have been used to treat a wide range of diseases [46]. Herbs and dietary supplements are gaining attention in cancer therapy [47]. *Picrorhiza kurroa*, a traditional Chinese herb, has been employed for treating liver [48] and respiratory disorders [49], chronic fever, and asthma [50]. The iridoid glycoside Picroside II (PII) is considered one of its active constituents, known for its hepatoprotective [51], anti-inflammatory [52] and neuroprotective properties [53]. Likewise, cucurbitacin B, extracted from various plant families, has shown strong cytotoxic effects against HER2–/ER+ breast cancer cells, RCC, HCC, and NSCLC [54]. Vinblastine and vincristine, derived from *Catharanthus roseus* (Vinca rosea), are potent cytotoxic agents. These vinca alkaloids are asymmetric dimers composed of vindoline and catharanthine. While vinblastine and vincristine are effective anti-cancer agents that interfere with cell cycle progression, vindoline alone lacks anticancer properties.

The selected plant compounds-Cucurbitacin B, Picroside, Vinblastine, Vindoline, And Vincristine-were evaluated using *in silico* docking analysis alongside two reference drugs, rapamycin and everolimus. The reference drug rapamycin interacts with FKBP12 through three van der Waals contacts (Trp59, Phe46, Ser38), three electrostatic interactions (Val155, Ile56, Asp37), and one hydrogen bond at Ile56. At the mTOR-FRB domain, rapamycin forms four van der Waals contacts (Ser124, Tyr127, Tyr26, Gln188) and two electrostatic interactions (Asp191, Thr187). Everolimus, on the other hand, binds FKBP12 via five van der Waals contacts (Phe46, Phe36, Ile90, Ile91, Gly129), five electrostatic contacts (Lys184, Asp191, Tyr26, Arg42, Tyr82), and one hydrogen bond with Ser124. At the mTOR-FRB domain, everolimus engages in four van der Waals contacts (Gly129, Tyr193, Trp190, Tyr193), three electrostatic contacts (Lys184, Ser124, Asp194), and one hydrogen bond with Tyr [26].

Among the phytochemicals studied, Picroside II demonstrated the highest affinity for FKBP [12], with a score of –12.134 kcal/Mol, compared to the rapamycin's -9.673 kcal/Mol. Additionally, Picroside II exhibited a lower binding energy (–61.595 kcal/mol) than rapamycin (-12.943 kcal/mol), indicating stronger binding affinity. At the FKBP12 domain, the protonated carboxyl group of Glu54 accepts a hydrogen bond, while Ile [56] donates one, despite isoleucine being a non-polar, hydrophobic residue, its backbone amino group serves as a hydrogen bond donor. Moreover, π–π interactions were observed between the aromatic rings of the ligand and Phe2108 within the mTOR-FRB domain.

These docking results are further supported by in vitro studies showing that Picroside II possess anti-metastatic and anti-angiogenic properties by suppressing the movement and invasion of breast cancer cells in humans. It also inhibited matrix metalloproteinase-9 (MMP-9) and downregulated gene expression in breast cancer cells [55]. Furthermore, compound's ability to mitigate cerebral ischemia - reperfusion injury through the downregulation of NF-κB and TLR4 may be integral to its anti-cancer effects [56].

Given its antioxidant, hepatoprotective, anti-allergic, anti-asthmatic, and anticancer activities, Picroside II derived from *P. kurroa* has great potential as a lead compound in drug development. It is also considered safe for oral consumption. However, additional in silico analyses including drug-likeness assessment, ADMET profiling, and pharmacophore studies along with in vitro and in vivo experiments, are essential to support and elaborate these findings.

## CONCLUSION

This study emphasizes the potential of naturally derived phytochemicals as mTOR inhibitors for breast cancer therapy, with Picroside II from *Picrorhiza kurroa* emerging as the most promising candidate. It exhibited superior binding affinity and stability to the FRB domain of mTOR, outperforming FDA-approved drug “rapamycin” in docking scores and binding energy. In vitro evidence supports its ability to inhibit cancer cell migration, invasion, and angiogenesis by downregulating MMP-9 and NF-κB pathways. Its established safety and traditional medicinal use further support its drug potential. With antioxidant, hepatoprotective, anti-inflammatory, and anticancer properties, Picroside II is a strong lead compound for targeted breast cancer treatment. However, further validation through *in silico* drug-likeness, ADMET profiling, pharmacophore modeling, and *in vitro*/*in vivo* studies is essential before clinical development.

## AUTHORS CONTRIBUTION STATEMENT

Ms. Krishnaveni G conducted the docking studies and drafted the manuscript. Dr. Asnet Mary J conceptualized and designed the entire study, overseeing the interpretation of the results. Dr. Renukadevi K contributed by reviewing and editing the manuscript. All authors collaborated in discussing the methodology and results, and jointly contributed to preparing the final manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors confirm that they have no conflicts of interest related to the publication of this paper.

## REFERENCES

1. Watkins EJ. Overview of breast cancer. Jaapa. 2019 Oct 1;32(10):13-7.
2. Baselga J. Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. The oncologist. 2011 Jan 1;16(S1):12-9.
3. López-Miranda E, Tolosa-Ortega P, Perelló-Martorell MA, Sánchez-Lorenzo L, Hinojo-González C, Servitja S, Recalde-Penabad S, Olier-Gárate C, Guerrero JA, García-Vicente S, Mina L. Human epidermal growth factor receptor 2 (HER2) expression dynamics between diagnosis and recurrence in patients with breast cancer using artificial intelligence and electronic health records: the RosHER study. ESMO Real World Data and Digital Oncology. 2025 Mar 1;7:100107.
4. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World journal of clinical oncology. 2014 Aug 10;5(3):412.

5. Orrantia-Borunda E, Anchondo-Nuñez P, Acuña-Aguilar LE, Gómez-Valles FO, Ramírez-Valdespino CA. Subtypes of breast cancer. Breast Cancer [Internet]. 2022 Aug 6.
6. Glaviano A, Foo AS, Lam HY, Yap KC, Jacot W, Jones RH, Eng H, Nair MG, Makvandi P, Geoerger B, Kulke MH. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Molecular cancer. 2023 Aug 18;22(1):138.
7. Hartkopf AD, Grischke EM, Brucker SY. Endocrine-resistant breast cancer: mechanisms and treatment. Breast care. 2020 Aug 21;15(4):347-54.
8. Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor α: a new model for anti-estrogen resistance. Journal of Biological Chemistry. 2001 Mar 30;276(13):9817-24.
9. Yardley DA. Combining mTOR inhibitors with chemotherapy and other targeted therapies in advanced breast cancer: rationale, clinical experience, and future directions. Breast cancer: basic and clinical research. 2013 Jan;7:BCBCR-S10071.
10. Mayer I. Role of mTOR inhibition in preventing resistance and restoring sensitivity to hormone-targeted and HER2-targeted therapies in breast cancer. Clinical advances in hematology & oncology: H&O. 2013 Apr;11(4):217.
11. Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22, 989), a new antifungal antibiotic I. taxonomy of the producing streptomycete and isolation of the active principle. The Journal of antibiotics. 1975;28(10):721-6.
12. Kennedy BK, Lamming DW. The mechanistic target of rapamycin: the grand conducTOR of metabolism and aging. Cell metabolism. 2016 Jun 14;23(6):990-1003.
13. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Molecular cell. 2002 Sep 1;10(3):457-68.
14. Saxton, R. A. & Sabatini, D. M. mTOR signaling in growth, metabolism, and disease. Cell 168, 960–976 (2017).
15. Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nature reviews Drug discovery. 2011 Nov;10(11):868-80.
16. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes & development. 2004 Aug 15;18(16):1926-45.
17. Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N. Dissecting the role of mTOR: lessons from mTOR inhibitors. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2010 Mar 1;1804(3):433-9.
18. Ballou LM, Selinger ES, Choi JY, Drueckhammer DG, Lin RZ. Inhibition of mammalian target of rapamycin signaling by 2-(morpholin-1-yl) pyrimido [2, 1-α] isoquinolin-4-one. Journal of Biological Chemistry. 2007 Aug 17;282(33):24463-70.
19. De Oliveira MA, e Martins FM, Wang Q, Sonis S, Demetri G, George S, Butrynski J, Treister NS. Clinical presentation and management of mTOR inhibitor-associated stomatitis. Oral oncology. 2011 Oct 1;47(10):998-1003.
20. Arriola Apelo SI, Lamming DW. Rapamycin: an InhibiTOR of aging emerges from the soil of Easter Island. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2016 Jul 1;71(7):841-9.
21. Li J, Kim SG, Blenis J. Rapamycin: one drug, many effects. Cell metabolism. 2014 Mar 4;19(3):373-9.
22. Peddi PF, Shatsky RA, Hurvitz SA. Noninfectious pneumonitis with the use of mTOR inhibitors in breast cancer. Cancer treatment reviews. 2014 Mar 1;40(2):320-6.
23. Rozengurt E, Soares HP, Sinnet-Smith J. Suppression of feedback loops mediated by PI3K/mTOR induces multiple overactivation of compensatory pathways: an unintended consequence leading to drug resistance. Molecular cancer therapeutics. 2014 Nov 1;13(11):2477-88.
24. Chandra S, Gahlot M, Choudhary AN, Palai S, de Almeida RS, de Vasconcelos JE, dos Santos FA, de Farias PA, Coutinho HD. Scientific evidences of anticancer potential of medicinal plants. Food Chemistry Advances. 2023 Oct 1;2:100239.
25. Yadav N, Deshmukh R, Mazumder R. A comprehensive review on the use of traditional Chinese medicine for cancer treatment. Pharmacological Research-Modern Chinese Medicine. 2024 Jun 1;11:100423.
26. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer: Targets and Therapy. 2019 Apr 10:151-64.
27. Society AC. Cancer facts & figures 2017. J. Consum. Health Internet. 2012;16:366-7.
28. Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: data from the Nurses' Health Study. American journal of epidemiology. 2000 Nov 15;152(10):950-64.
29. Collaborative Group on Hormonal Factors in Breast Cancer, 2001. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58 209 women with breast cancer and 101 986 women without the disease. The Lancet, 358(9291), pp.1389-1399.
30. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Personal habits and indoor combustions. Volume 100 E. A review of human carcinogens. IARC monographs on the evaluation of carcinogenic risks to humans. 2012;100(PT E):1.

31. Baer HJ, Tworoger SS, Hankinson SE, Willett WC. Body fatness at young ages and risk of breast cancer throughout life. American journal of epidemiology. 2010 Jun 1;171(11):1183-94.
32. International Agency for Research on Cancer. IARC handbooks of cancer prevention. The Agency; 2005.
33. Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. BioMed research international. 2013;2013(1):747318.
34. Kenemans P, Verstraeten RA, Verheijen RH. Oncogenic pathways in hereditary and sporadic breast cancer. Maturitas. 2004 Sep 24;49(1):34-43.
35. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World journal of clinical oncology. 2014 Aug 10;5(3):412.
36. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: Past, present and future. InSeminars in cancer biology 2018 Oct 1 (Vol. 52, pp. 56-73). Academic Press.
37. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, Van De Rijn M, Jeffrey SS, Thorsen T. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences. 2001 Sep 11;98(19):10869-74.
38. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. 2012 Sep *Nature* 490: 61–70.
39. Zhang W, Haines BB, Efferson C, Zhu J, Ware C, Kunii K, Tammam J, Angagaw M, Hinton MC, Keilhack H, Paweletz CP. Evidence of mTOR activation by an AKT-independent mechanism provides support for the combined treatment of PTEN-deficient prostate tumors with mTOR and AKT inhibitors. Translational Oncology. 2012 Dec 1;5(6):422-9.
40. Bruhn MA, Pearson RB, Hannan RD, Sheppard KE. AKT-independent PI3-K signaling in cancer–emerging role for SGK3. Cancer management and research. 2013 Aug 26:281-92.
41. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011 Mar 4;144(5):646-74.
42. Lien EC, Dibble CC, Toker A. PI3K signaling in cancer: beyond AKT. Current opinion in cell biology. 2017 Apr 1;45:62-71.
43. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007 Jun 29;129(7):1261-74.
44. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex interacting with binding domain of human FRAP. Science. 1996 Jul 12;273(5272):239-42.
45. Royce ME, Osman D. Everolimus in the treatment of metastatic breast cancer. Breast cancer: basic and clinical research. 2015 Jan;9:BCBCR-S29268.
46. Dar RA, Shahnawaz M, Qazi PH. General overview of medicinal plants: A review. The journal of phytopharmacology. 2017;6(6):349-51.
47. Hou YN, Deng G, Mao JJ. Practical application of “about herbs” website: herbs and dietary supplement use in oncology settings. The Cancer Journal. 2019 Sep 1;25(5):357-66.
48. Scott Luper ND. A review of plants used in the treatment of liver disease: part two. Alternative Medicine Review. 1999;4(3):178-89.
49. Dwivedi Y, Rastogi R, Garg NK, Dhawan BN. Picroliv and its components kutkoside and picroside I protect liver against galactosamine-induced damage in rats. Pharmacology & toxicology. 1992 Nov;71(5):383-7.
50. Almeleebia TM, Alsayari A, Wahab S. Pharmacological and clinical efficacy of Picrorhiza kurroa and its secondary metabolites: A comprehensive review. Molecules. 2022 Nov 29;27(23):8316.
51. Gao H, ZHOU YW. Inhibitory effect of picroside II on hepatocyte apoptosis 1. Acta Pharmacologica Sinica. 2005 Jun;26(6):729-36.
52. Huang Y, Zhou M, Li C, Chen Y, Fang W, Xu G, Shi X. Picroside II protects against sepsis via suppressing inflammation in mice. American journal of translational research. 2016 Dec 15;8(12):5519.
53. Wang Y, Chen R, Yang Z, Wen Q, Cao X, Zhao N, Yan J. Protective effects of polysaccharides in neurodegenerative diseases. Frontiers in Aging Neuroscience. 2022 Jul 4;14:917629.
54. Clericuzio M, Mella M, Vita-Finzi P, Zema M, Vidari G. Cucurbitane Triterpenoids from Leucopaxillus g entianeus. Journal of natural products. 2004 Nov 29;67(11):1823-8.
55. Lou C, Xu X, Chen Y, Zhao H. Alisol A suppresses proliferation, migration, and invasion in human breast cancer MDA-MB-231 cells. Molecules. 2019 Oct 10;24(20):3651.
56. Wang Y, Huang J, Ma Y, Tang G, Liu Y, Chen X, Zhang Z, Zeng L, Wang Y, Ouyang YB, Yang GY. MicroRNA-29b is a therapeutic target in cerebral ischemia associated with aquaporin 4. Journal of Cerebral Blood Flow & Metabolism. 2015 Dec;35(12):1977-84.