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Anti-Breast Cancer and Pharmacokinetic Prediction of Isorhamnetin, Glucocapparine Capparisine A And B From *Capparis Spinosa*

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ABSTRACT

The discovery of new natural anticancer agents is considered a remarkable solution to prevent drug resistance and metastasis in breast cancer patients. The *Capparis spinosa* plant is widely known for its antioxidant and other therapeutic effects. This study aims to evaluate anti-breast cancer activity targeting Akt1 and HIF1A proteins of several phytochemicals in *Capparis spinosa* through *in silico* methods and their pharmacokinetic prediction. This research is an *in-silico study* involving Bioinformatics (PASS Analysis with STITCH & STRING), Molecular Docking, and pkCSM Analysis. According to bioinformatics methods, AKT1 and HIF1A were obtained as potential protein targets. In molecular docking to AKT1 protein, the docking score obtained for Isorhamnetin, Glucocapparine, Capparisine A, Capparisine B, and Ipatasertib as control were -6.2 kcal/mol, -5.3 kcal/mol, -4.9 kcal/mol, -4.9 kcal/mol, and -6.2 kcal/mol respectively. Meanwhile, the docking score for HIF1A protein for Isorhamnetin, Glucocapparine, Capparisine A, Capparisine B, and 2-methoxy estradiol as control was -5.2 kcal/mol, -4.7 kcal/mol, -4.4 kcal/mol, -4.3 kcal/mol, and -4.7 kcal/mol. The scores for each compound were like the controls in both proteins, indicating that the analyzed phytochemicals of *Capparis spinosa* have potential anti-breast cancer properties. Pharmacokinetic prediction for absorption, distribution, metabolism, excretion, and toxicity (ADMET) is also provided to help further studies and development for the compounds as anticancer drugs. This study provided data from in silico methods regarding anti-breast cancer supported with pharmacokinetic activities for Isorhamnetin, Glucocapparine Capparisine A, and Capparisine B, which will be helpful as a reference for other advanced research in the future.

Keywords: *Breast Cancer, Capparis spinosa, Bioinformatics, Molecular Docking, pkCSM.*

INTRODUCTION

Breast cancer has a remarkable rank among the most widespread and deadly illnesses on a global scale. According to the World Health Organization, global statistics show that in 2020, breast cancer was diagnosed in approximately 2.3 million women, resulting in approximately 685000 deaths (Sung et al., 2021). Research has indicated that the spreading and proliferation of breast cancer cells at a secondary area in the body contribute significantly to the mortality rate, especially considering that 81% of breast cancers are invasive as it is suggested that death occurs from spreading the cancer cells to the other vital organs like liver, lung, and brain which will result in impairing their function (Department of Defence Breast Cancer Research Program, 2021; American Cancer Society, 2022). Furthermore, chemotherapy resistance results in the failure of anti-breast cancer chemotherapy, leading to a higher mortality rate. This condition is defined as the ability of cancer cells to avoid the effects of the chemotherapeutic agent, resulting in low efficacy of the drug to produce a helpful response in the treatment, which can arise due to factors that are related to the tumour or the host (Jia et al., 2019).

Metastasis is a joint event in malignant tumours usually associated with a bad prognosis and low survival rate. It can be defined as the process of spreading tumour cells from the primary site to the secondary site by invasion. Tumour metastasis consists of multi-steps including the development of rapidly proliferating clones of cancer cells that have the essential biological properties to complete the steps involved in the metastasis, tumour angiogenesis, and tumour cell loosening due to the inactivation of cell adhesion molecules (CAMs) such as epithelial-cadherin (E-cadherin) which will be attached to the extracellular Matrix (ECM) and interact with it to degrade the ECM resulted from overexpression of proteases and matrix-degrading enzymes such as Matrix metalloproteinases (MMPs) that will firstly degraded basement membrane of the tumour itself, then create a road for tumour cells through the interstitial matrix, and finally degrade the basement membrane of the vessel wall which results in cancer metastasis (Mohan, 2018).

A study has shown that Matrix metalloproteinases (MMPs), which are ECM-degradable enzymes, extend beyond cancer cells' development, invasion, and spread. Furthermore, the activation of various signalling pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt pathway, is closely associated with the expression of MMPs, which regulate it at the transcriptional level. The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is involved in regulating multiple tumorigenic functions within breast cancer including regulating cell proliferation, survival, angiogenesis, metabolism, drug resistance, expansion, and metastasis (Marinescu et al., 2020; Janku et al., 2018).

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Hypoxia-inducible factor-1α (HIF-1α) is also expressed in breast cancer as a primary regulator for cancer cells to adapt to hypoxic condition and play critical roles in many significant molecular activities that are significant predictor of poor prognosis such as tumour invasion, metastasis, and resistance to therapeutic drugs (Luo et al., 2022; Peiro et al., 2019). Therefore, the properties of PI3K/Akt and HIF-1α pathways position them as an attractive candidate for cancer therapy. Also, targeting and inhibiting these pathways may be a promising strategy in cancer treatment, especially for cancer cells with a high propensity for metastasis and show resistance to conventional chemotherapy.

Research and development of novel anticancer agents from the herbal plant are considered potential solutions for those problems as the history of anticancer drug discovery is marked by natural products. In addition, a significant number of commonly used cancer-fighting drugs are derived from natural origin. According to scientific investigation, nearly 25% of all new anticancer drugs that gained approval from 1981 to 2019 originated from natural sources (Huang et al., 2021). Moreover, 80% of FDA-approved drugs utilized in cancer treatment are produced from natural sources and their derivatives (Yuan et al., 2023). In addition, utilizing plant-based raw materials containing appropriate bioactive compounds and administering them at appropriate doses has proven to be a more efficient and safer approach to treating various diseases (Hameed et al., 2021).

Capparis spinosa is a plant distributed worldwide and is known as a herbal drug with significant values, especially after many studies have shown that its active constituent is responsible for many biological activities. Previous studies investigated that *C. spinosa* plants have many active constituents from different chemical families, such as flavonoids, terpenoids, alkaloids, phenolic acids, flavonoids, coumarins, resins, glucosinolates, tannins, saponins, organic acids, and fatty acids. Due to this diversity in the active constituent, it is currently known to have many pharmacological activities, such as antioxidative activities, anticarcinogenic, Anti-inflammatory, Immunomodulatory activity, etc (Al-Azawi et al., 2018; Hameed et al., 2021),

This study aims to evaluate anti-breast cancer activity targeting Akt1 and HIF1A proteins of several phytochemicals in Capparis spinosa through in silico methods and their pharmacokinetic prediction.

LITERATURE REVIEW

Breast cancer is a heterogeneous disease in which cells in breast tissue are modified and undergo rapid uncontrolled division, as well as genetic and epigenetic alterations, resulting in the development of a lump or mass. Cancer chemotherapy resistance is a problem that threatens many cancer patients' lives, as the cancer cells will have the ability to evade the effects of chemotherapeutics resulting in low efficacy of the therapeutic agent to produce a useful response in the treatment resulting in treatment failure and indirect enhancing tumour progression. Scientists and researchers have started to look for new targets that can inhibit or affect cancer cells and limit their metastasis and progression.

Many studies show that the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the Hypoxia-inducible factor-1α (HIF-1α) are attractive targets in cancer therapy. The phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a significant role in cell development, proliferation, cellular metabolism, and survival (Martorana et al., 2021).

AKT, also called protein kinase B, is a critical effector of the phosphoinositide3-kinase (PI3K) intracellular pathway, which controls multiple vital cellular functions, including cell development, rapid reproduction, metabolism, and survival. In mammals, AKT exists in three forms, namely Akt1/protein kinase Bα (PKBα), Akt2/protein Kinase Bβ (PKBβ), and Akt3/protein kinase Bγ (PKBγ) which are encoded by separate genes with highly conserved cellular homologs and covered by protein structure. The three AKT proteins share a common structure feature, consisting of three functional domains: an N-terminal with pleckstrin homology (PH) domain, a large central kinase domain and a C-terminus regulatory domain (RD) (Fig. 1) (Coleman et al., 2021; Martonara et al., 2021). Among the three isoforms, Akt1 and Akt2 are widely and abundantly expressed isoforms and are present in various tissues. In comparison, Akt3 is commonly found in neuronal tissues (Hinz & Jücker, 2019). Increased activation of the three Akt types can be involved in the cancer environment and advancement, as exhibited in various types of cancers, including breast, pancreatic, ovarian, and prostate cancers, among others (Song et al., 2019). Akt1 is implicated in cell growth and proliferation in tumour cells, supports cancer initiation, inhibits apoptosis, and promotes cell survival. At the same time, Akt2 controls cytoskeleton dynamics, regulates metabolism, and facilitates cell metastasis and invasion. The involvement of Akt3 in tumours remains a topic of debate, as its exact role is not yet fully understood. However, a hypothesis suggests it may promote cell proliferation and participate in cell growth mechanisms, possibly in collaboration with Akt1 (Hinz & Jücker, 2019).

Fig 1. AKT structure

Signalling initiation in the PI3K/Akt pathway involves various mechanisms and predominantly originates from the cell membrane, beginning with the activation of tyrosine kinases receptor (TKR), G-protein coupled receptors and other receptors, either by direct or indirect interaction through phosphorylation of adaptor molecules. Activated PI3K results in an elevation in the amount of phosphatidylinositol trisphosphate (PIP3) through phosphorylates of a membrane phospholipid called

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phosphatidylinositol bisphosphate (PIP2) (Park and Ma, 2018). PIP3 binds to AKT at the PH domain, enabling phosphoinositide-dependent kinase-1 (PDK1) to direct AKT towards the cell membrane, where AKT is located. Upon reaching the cell membrane, double phosphorylation of AKT occurs. one by PDK1 which phosphorylates Akt at kinas domain at Threonin308 (T308) and another done by the mTOR complex 2 (mammalian target of rapamycin complex2 / mTORC2) which phosphorylate Akt on the regulatory domain at serine473 (S473), In addition to mTORC2 studies have found that various Kinases also can phosphorylate Akt at Ser4723 such as DNA-dependent protein kinase (DNA-PK) and integrin-linked kinase (ILK), resulting in its full activation. (Martorana et al., 2021; Nitulescu et al., 2018). Once activated, Akt breaks the PIP3 binding and participates in the phosphorylation of an enormous number (>100) of protein substrates located in the nucleus, cell membrane, mitochondria or cytosol that carry on the signal to elicit its effects (Hoxhaj & Manning, 2020).

Major effectors downstream of Akt include inhibition of AS160, a negative regulator of Glute-4 translocation, which moves to the plasma membrane after inhibition of AS160 and allows glucose to enter the cell and undergo glycolysis. Akt also suppresses tuberous sclerosis complex 1 and 2 (TSC1/2) formation and effectively prevents Rheb activation. Rheb acts as a protein activator of mTORC2, which subsequently, triggers S6kinase (S6k) phosphorylation, which activates ribosomal protein at S6 through phosphorylation which increases and promotes protein synthesis and cell proliferation. In addition, activating mTORC2 can reduce autophagy, a critical mechanism that moves the cells toward programmed cell death (Yu C et al., 2018). Also, Akt can control cell survival and proliferation through the inhibition of signals that promote cell death, such as those mediated by Forkhead box O (FOXO) transcription factors and Bad. The role of FOXO is to stimulate the duplication of specific genes, ultimately enhancing the termination of cell cycle, apoptosis, and cellular oxidative stress, ensuring metabolic stability.

Furthermore, when FOXO transcription becomes inactive, they are expelled from the nucleus to the cell cytoplasm, where they break down, ultimately supporting the survival of the cells. Also, inhibition of FOXO will result in cell cycle progression as inhibits the transcription cyclin-dependent kinase (CDK) inhibitors p27 and p21. It also blocks the external cell death pathway (Nitulescu et al., 2018). Glycogen synthase kinase 3 (GSK3), which is responsible for the phosphorylation of glycogen synthase (GS) to an inactive form, is seen that Akt could inhibit it and, thus, promote glycogen synthesis. Furthermore, multiple biochemical processes within the tumour are altered by the presence of GSK3. Studies have shown that GSK3 plays a role in tumour proliferation and actively contributes to metabolic processes in the tumour. It has been proposed that apoptosis is heightened, and the framework of membrane lipids undergo modification when GSK3 inhibitor IX are present (Acikgoz et al., 2019). In addition, Akt can activate ATP-citrate lyase, which is essential for fatty acid synthesis.

The entire pathway undergoes a negative regulation, facilitated by the activity of phosphatases, such as the phosphatase and tensin homolog (PTEN) that down-regulate PIP3 through a transformation of PIP3 to PIP2. PHLPP is another phosphatase that negatively regulates this pathway through the dephosphorylation of serene473. Furthermore, Protein phosphatase 2A (PP2A) is another negative regulator that dephosphorylation of Threonin308 (Lee et al., 2018) (Figure 2).

The importance of AKT includes its ability to regulate various essential cellular processes such as controlling cell size, enhancing the cellular division cycle, managing glucose metabolism levels, stabilizing genes, facilitating duplication activities and stimulating protein synthesis and lipid and glycogen production. Moreover, AKT contributes to new blood vessel formation processes and acts as a mediator for cellular growth factors to support cell survival while inhibiting regulated cell death (Nitulescu et al., 2018).

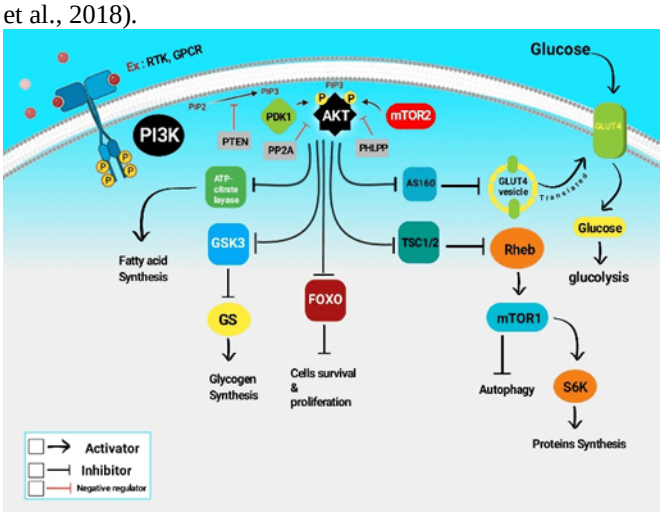

Fig 2. AKT signalling pathway

A study has shown that abnormalities that occur in Akt activation are highly oncogenic and can take place by several mechanisms and arise from various causes, such as mutation or expansion of AKT, through genomic modification at a different level in the pathway as researches indicated that in around 40% of cancer types, abnormalities in the PI3K/AKT pathway have been noted (Coleman et al., 2021). Other studies have shown that gene mutations and alterations in cell signalling pathways that occur in the PI3K/AKT pathway contribute to the onset and progression of breast cancer (Yuan et al., 2023). Mutations in the PI3K, explicitly focusing on the PI3K catalytic alpha subunit (PIK3CA), have been reported in breast cancer cases at a rate ranging from 9–45%. Mutation in PTEN occurs in approximately 13–35%, whereas AKT substitutions occur with lower frequency at 2-4%, and amplification is also less prevalent (5–10%) (Martorana et al., 2021). A study has estimated the frequency of PI3K/ Akt pathway activation and variation again by breast cancer subtypes, as HR+ breast cancer shows the highest value or rate in PI3K/AKT

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pathway hyperactivation (up to 50%); this activation is predominantly carried out by PIK3CA point mutations, where ER+ metastatic breast cancer show 5–10% hyperactivation mainly by somatic PTEN mutations, it also found that the occurrence of ER+ breast cancer due to mutations in AKT1 is estimated to be around 7%, in addition, in advanced triple-negative breast cancer (TNBC), there is a presence of activating mutations in both PIK3CA and AKT1, along with inactivating PTEN mutations occurring in rate of 25%–30%.

Furthermore, breast cancer may show deregulation in human epidermal growth factor receptor 2 (HER2), improving resistance toward antiHER2 agents. Due to the significant rate of Akt dysregulation, AKT inhibitors hold great promise as an interesting and effective category of anticancer drugs, particularly in targeting breast cancer (Coleman et al., 2021). On the other hand, studies indicate that the overactivation of the PI3K/AKT pathway can contribute to cancer formation, while suppression of the PI3K–AKT pathway may induce cell death. Yuan et al. (2023) report that several scientific investigations have demonstrated that various naturally occurring plant sources can suppress the growth of breast cancer cells through the modulation of the PI3K–AKT pathway in mice model studies. According to a different study, dysregulation of the PI3K-AKT pathway is a feature shared by triple-negative tumours, HER2-amplified illness, and hormone receptor-positive (HR+) disease types of breast cancer. For several breast cancer subtypes, especially those that resist traditional therapies, selective targeting of AKT thus presents an appealing therapeutic approach (Martorana et al., 2021).

The deregulation of the PI3K/Akt signalling pathway not only bestows carcinogenic properties but also assumes an essential role in conferring resistance to chemotherapeutic drugs. Multiple studies have demonstrated that IGF1R/p110β/AKT/mTOR signalling pathways contribute to developing resistance to BYL-719 in breast tumours with PIK3CA mutations. A recent study by He et al. (2021) has provided further evidence supporting the efficacy of targeting proteins within the PI3K/Akt signalling pathway to overcome medication resistance in cancer treatment.

Numerous studies have established a correlation between the upregulation of Akt in cancer cells and the emergence of resistance to radiation and chemotherapy drugs. Moreover, previous research has demonstrated that the upregulation of the PI3K/Akt signalling pathway triggers several pathways' activation in diverse breast cancer subtypes of several pathways in diverse breast cancer variants. These pathways, which subsequently result in the development of chemotherapy resistance, encompass Notch, WNT/β-catenin, mitogen-activated protein kinase (MAPK), and nuclear factor-κB. Research has shown that the PI3K/Akt signalling pathway is vital in giving cancer cells chemoresistance in the tumour microenvironment. Multiple mechanisms, including the activation of drug efflux pumps, including multidrug resistance (MDR) proteins like p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), are involved in mediating this resistance.

Additionally, this signalling pathway influences immune responses, activates antioxidant signalling, and inhibits programmed cell death by activating nuclear factor erythroid 2-related factor 2 (Nrf2), a cellular defence mechanism against chemotherapy. Moreover, it activates survival signalling pathways in various types of human breast cancer (Kaboli, 2021). The findings of these studies suggest that selectively targeting the different constituents of the PI3K/AKT pathway appears to be an appropriate strategy for addressing the therapy of diverse subtypes of breast cancer.

Cancer cells exhibit faster growth and metabolism rates. They appear with a distinct glycometabolic pathway, separating them from normal, healthy cells. Aerobic glycolysis can generate numerous metabolites concomitant with the rapid production of a substantial amount of adenosine triphosphate (ATP). According to Kobliakov (2019), the presence of certain metabolites and energy sources has the potential to accelerate cancer progression. The regulation of aerobic glycolysis in tumour cells is regulated by the transcription factor HIF-1a, which is amplified in response to hypoxic conditions. When the partial pressure of oxygen falls below 10 mm, it leads to the upregulation of HIF-1a, which could stimulate glycolysis (Yang et al., 2022).

Hyperdimer transcription factors, known as hypoxia-inducible factors (HIFs), are oxygen-dependent. This factor controls the reaction of molecules to insufficient levels of cellular oxygen. HIF comprises two subunits, one of which is an oxygen-sensitive and labile alpha subunit (HIF-1α, HIF2±, HIF3±). Additionally, there is a stable beta subunit (HIF-1β) that is unaffected by oxygen (Mendoza et al., 2023).

Under typical circumstances, the regulation of HIF1-α primarily occurs through post-translational changes, specifically governed by two proline residues. These proline residues are part of O2-dependent prolyl-4-hydroxylases (PHDs), ultimately resulting in the hydroxylation of HIF-1α. The E3 ubiquitin ligase complex known as von Hippel-Lindau (VHL) is responsible for recognizing the hydroxylation of HIF1. This recognition triggers the process of polyubiquitination, leading to the eventual destruction of HIF-1 by the proteasome (32, 34). nevertheless, in the absence of oxygen or other essential cofactors, the activity of the PHDs is inhibited, leading to the generation of HIF-1 inside the cellular environment (Jeknic et al., 2022).

During the hypoxia condition, the HIF-1α is stabilized, leading to a complex formation with the HIF-1β - subunit, which affects the transcription of genes that supported the cell adaptation to low oxygen condition. The two isoforms of the alpha subunit, HIF-1α and HIF2α, are the primary transcription regulator; meanwhile, the HIF-3α antagonized the other factors. This adaptation involved

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cellular responses such as angiogenesis, erythropoiesis, and energy metabolism (Mendoza et al., 2023).

The two domains that make HIFα are the N-terminal transactivation domain (TAD), which is mainly responsible for stabilizing HIFα, and the C-terminal transactivation domain (TAD), which interacts with the transcriptional co-activator protein/P300 under hypoxic conditions to regulate HIF-α transcription. According to Infantino et al. (2021), HIF-α degradation by the ubiquitin-proteasome pathway is facilitated by the basic helix-loop-helix (bHLH), oxygen-dependent degradation domain (ODD), and Per-Arnt-Sim (PAS) domain. The inter-TAD sections correspond to inhibitory domains, which play a vital function in inhibiting the TAD's transcriptional activation, which is accomplished by preventing the TAD from binding to other transcription factors.

The HIF-1β subunit does not possess the ODD and N-TAD domains and exclusively possesses the C-TAD domain, resulting in structural distinctions from HIFα. These structural differences are manifested in the functional characteristics of HIF-1β (Infantino et al., 2021).

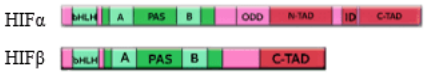

Fig 3. HIF Structure

Research findings indicate that HIF-1α has a role in the development of drug resistance in breast cancer because it tends to be resistant to conventional therapies through a variety of signalling pathways, such as drug outflow, tumour stem cell abundance, autophagy, and apoptosis (Jin et al., 2019). Based on the earlier information, HIF-1α has emerged as a compelling subject of interest in the field of cancer treatment due to its role as a significant transcriptional regulator of the hypoxic response. Moreover, it exerts a considerable impact on the facilitation of angiogenesis, glucose metabolism, and cancer cell survival. Additionally, its direct association with an unfavourable prognosis further underscores its relevance in predicting disease outcomes.

Since the beginning, medicinal plants and natural products have been used as alternative cancer treatments due to patients' perception that they are safer and have fewer side effects and toxicity. A comprehensive understanding of plant and natural products' intricate biological processes has recently been discovered. These substances exhibit complicated actions within cells, acting upon many specific proteins to restore cellular homeostasis. Moreover, the molecule derived from natural sources possesses distinct attributes, rendering its precise activities, potential method of action, potential targets, and synergistic effects with other compounds within cells undetermined. In addition, it should be noted that there exists a scarcity of empirical evidence about phytochemical-protein interactions (PCPIs). Consequently, the integration of new technology is necessary to create natural drug products effectively (Najmi et al., 2022; Huang et al., 2018). Furthermore, there is a constant drive among the scientific community to discover novel pharmaceuticals and optimize drug development processes to achieve medications with enhanced efficacy and minimal adverse effects.

The drug discovery and development process necessitate identifying a limited number of promising compounds from various chemical constituents inside medicinal plants. Screening and evaluating an extensive compound library using laboratory assays might pose significant challenges. Implementing computer-aided screening prior to laboratory assay has been suggested as a viable strategy (Wilson et al., 2020). Recently, there has been a growing trend in the utilization of in silico methodologies in medicinal plant research. These methods have proven to be suitable for the identification of drug targets, the discovery of hit compounds, the investigation of multi-targeted actions, and other important aspects related to the process of drug discovery and the development of natural products (Briones et al., 2021). As mentioned before, the drug discovery and development process require a careful selection of a few potential components from the vast number of chemical constituents present in medicinal plants. Hence, Molecular docking is an effective tool in this process, assisting in identifying effective or lead compounds within these plant compounds (Alshahrani et al., 2021). Numerous studies have provided evidence indicating that a significant proportion of failures in clinical trials within drug discovery and development can be attributed to suboptimal pharmacokinetics.

Consequently, it is imperative to prioritize considering and estimating pharmacokinetics at the earliest stages of the drug discovery process. Furthermore, it is advisable to employ early prediction techniques to assess the potential of phytochemicals to reach the intended target site effectively. Utilizing in silico methods can enhance the drug discovery and development process while concurrently mitigating the frequency of failures in clinical trials. This reason is primarily due to the availability of established tools designed explicitly for this purpose, as highlighted by Chang et al. (2023).

The utilization of in silico methods holds significant potential in facilitating the rapid development and precise identification of drug targets and in the prediction of effective drug discovery methods.

METHOD

This study was conducted through *in silico* methods involving bioinformatics such as PASS (Prediction of Activity Spectra for Substances) and STITCH-STRING combination to determine each compound activity and protein target related to breast cancer. PASS analysis was carried out through the website http://www.way2drug.com/PASSOnline/. The SMILES code of each compound is inputted then pharmacological activities along with Pa (Probable activity) and Pi (probable inactivity) values are obtained. STITCH-STRING methods were conducted by combining several websites: http://stitch.embl.de/ (to get direct target protein that binds to the analyzed compound), https://string-db.org/ (to get indirect target proteins that bind to the analyzed compound),

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https://pubmed.ncbi.nlm.nih.gov/ (to get proteins that regulated breast cancer), and http://www.interactivenn.net. (to discover direct and indirect target proteins from the compound that regulated breast cancer development through the Venn diagram). The result from the diagram was then analyzed to get a protein-protein interactions network (PPI) using the previous string website combined with the Cytoscape application. From PPI, hubb proteins or the protein with the highest degree score is obtained. Two proteins from both bioinformatic methods were then analyzed as target proteins of compounds through molecular docking to evaluate the binding affinity, represented by affinity in kcal/mol. This procedure involved *Autodock Vina, Autodock Tools, and DS Visualizer applications.* Whole compounds are then analyzed through the pkCSM method using the website http://biosig.unimelb.edu.au/pkcsm/prediction. to obtain pharmacokinetic prediction such as absorption, distribution, metabolism, excretion, and toxicity of a compound inside the body.

Organized procedures would significantly support the field of natural products research. In response to this requirement, we constructed an innovative methodology that integrates several methodologies for estimating and visualizing the cellular impacts of medicinal plant-derived chemicals in disease therapeutics (Fig.4)

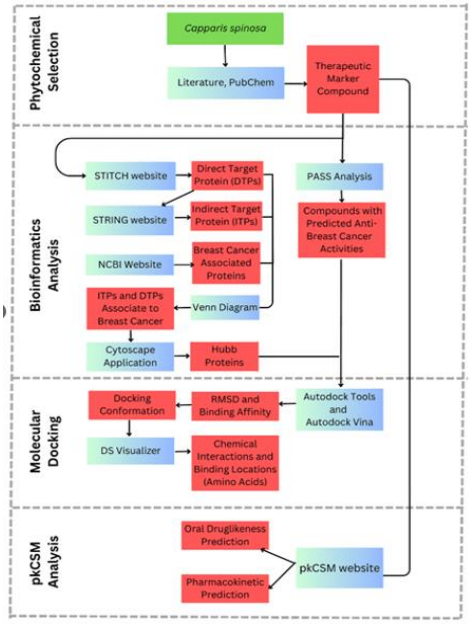
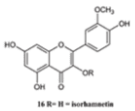
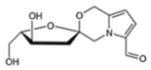
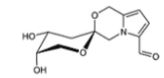


Fig 4. Schematic Visualization of Research Methods

RESULT AND DISCUSSION

The following list of chemical classes, compounds, and analytical techniques is based on the literature review that was done and utilized to figure out the components that naturally occur in C. spinosa and that are employed in this study:

Table 1. Phytochemical Selection Result

Compounds	Chemical classes	Methods on analysis	Chemical structure	Refrense
Isorhamnetin	Flavonoid	HPLC/UV–vis-DAD/ESI-MS		Siracusa et al., 2011
Glucocapparin	Glucosinolate	Paper chromatography	 26 glucocapparin	Ahmed et al., 1972
Capparisine A	Alkaloid	Silica gel column chromatography, reversed-phase HPLC, NMR, X-ray crystallographic analysis		Yang et al., 2010
Capparisine B	Alkaloid	Silica gel column chromatography, reversed-phase HPLC, NMR, X-ray crystallographic analysis		Yang et al., 2010

According to PASS analysis, all the compounds have several pharmacological activities related to breast cancer.

Table 2. PASS Analysis Result

Compound	Breast Cancer-Related Pharmacological Activities (Probable Activity, Probable Inactivity Value)
Capparisine A	HIF1A expression inhibitor (0.743, 0.016); Cytostatic (0.378, 0.048
Capparisine B	HIF1A expression inhibitor (0.627, 0.029); Antineoplastoic (0.531, 0.062)
Glucocapparine	Antineoplastic (0.866, 0.005); Antimetastatic (0.426, 0.039)
Isorhamentin	HIF1A expression inhibitor (0.963, 0.003); Antineoplastic (0.803, 0.011); Chemopreventive (0.772, 0.004); Breast cancer-resistant protein inhibitor (0.687, 0.001); Antineoplastic (breast cancer) (0.600, 0.010).

Isorhamnetin, Capparisine A and B showed inhibitory activity for HIF1A expression. HIF1A or Hypoxia Inducible Factors is one of the genes that regulate the ability of solid tumours such as breast cancer to deal with decreased O2 condition due to massive cell proliferation). HIF-1a can cause tumour cells to engage in anaerobic glycolysis, generate angiogenesis, encourage tumour cell growth, invasion, and migration, and result in drug resistance (Yong et al., 2022). Glucocapparine does not show any specific protein target in breast cancer yet exhibits the highest antineoplastic probable activity value (0.866).

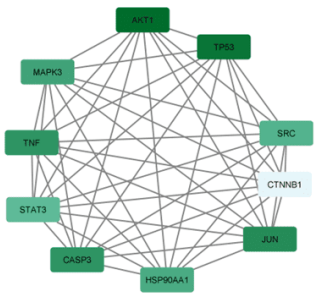


Fig 5. Protein-Protein Interaction obtained from STITCH-STRING Analysis

The bioinformatics was also conducted through STITCH-STRING methods, which resulted in protein-

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protein interaction after being visualized by the Cytsocape application.

Table 3. Hub Proteins obtained from STITCH-STRING Method

Protein	Degree Score
AKT1	69
TP53	68
JUN	65
CASP3	64
TNF	64
MAPK3	62
HSP90AA1	61
SRC	60
STAT3	59
CTNNB1	58

STITCH-STRING method exhibited AKT1 as a protein with the highest degree score. According to Basar et al. (2022), both directly linked and indirectly related hub proteins and genes are connected by the Protein-Protein Interaction (PPI) network. AKT1, as a hubb protein, is assumed to have a vital role among proteins analyzed in PPI. Thus, this protein could be a target protein in the next step.

Two proteins that potentially can be bound to compound according to bioinformatics, both PASS and STITC-STRING methods, are HIF1A and AKT1. HIF1A is chosen to be a target protein due to the high potential of most of the compounds to bind to this gene, according to PASS Analysis. Meanwhile, according to the bioinformatics method, AKT1 was obtained as the hubb protein with the highest degree score.

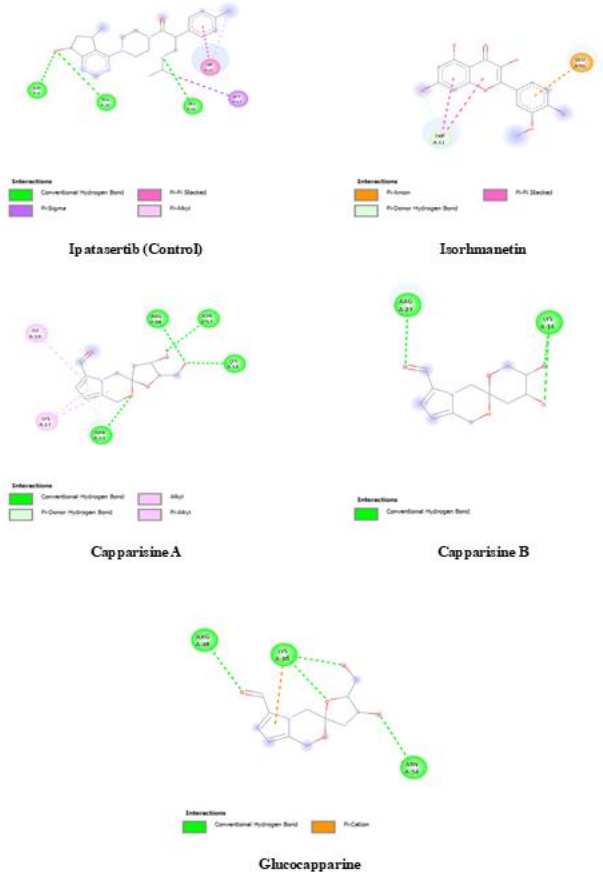

Fig 6. The 2-dimensional visualization output from the molecular docking process conducted on AKT1.

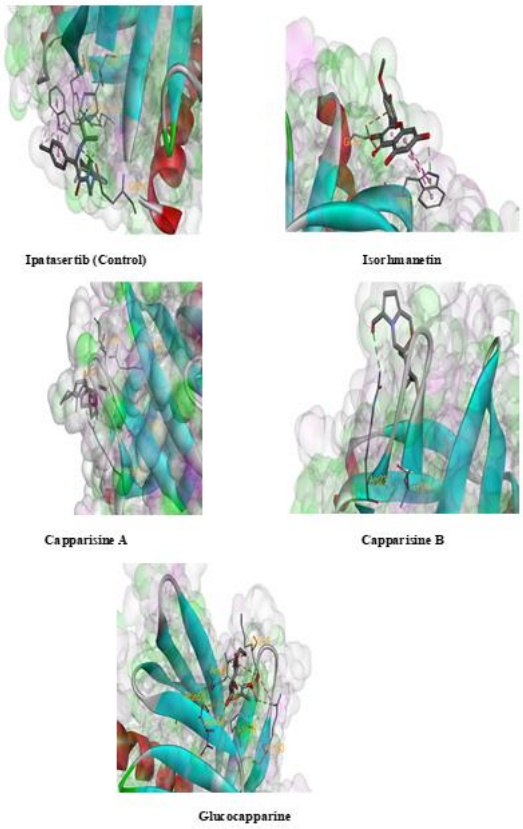

Fig 7. The 3- 3-dimensional visualization output from the molecular docking process on AKT1.

Table 4. Output of Molecular Docking in AKT1

Compounds Name	RMSD	Afinity (Kcal/Mol)ss	Position of Chemical Interactions		
			Hydrogen Bonds	Electrostatic Interactions	Hydrophobic Interactions
Ipatasertib (Control)	1.640	-6.2	GLU: 9, TYR:26, GLU:91	TRP:11	TRP:11, HIS:13
Isorhamnetin	0.771	-6.2	VAL:90, HIS:13	GLU: 91, TRP:11	TRP:11
Capparisine A	1.782	-4.9	ARG:86, ASN:53, ARG:23, LYS:14	-	ILE:19, LYS: 17
Capparisine B	1.866	-4/9	ARG: 23, LYS: 14	-	-
Glucocapparine	1.787	-5.3	ASN: 54, ARG: 48, LYS: 30	LYS: 30	-

The affinity of each compound towards the proteins is obtained from the bioinformatics method and then evaluated via molecular docking. The result showed that the highest affinity towards AKT1 protein was observed in Isorhamentin (-6.2 kcal/mol), which has the same value as Ipatasertib as the control. Ipatasertib is an inhibitor that acts competitively by binding to the active site of AKT1 to interfere with the AKT pathway and cell cycle (Shariati & Bernstam, 2019). Through visualization in the DS visualizer, Isorhamnetin appeared to form hydrogen bonds with several amino acids in AKT1, namely VAL 90 and HIS 13; other chemical interactions, such as electrostatic and hydrophobic interactions, were also predicted. The whole result can be seen in Table 1.

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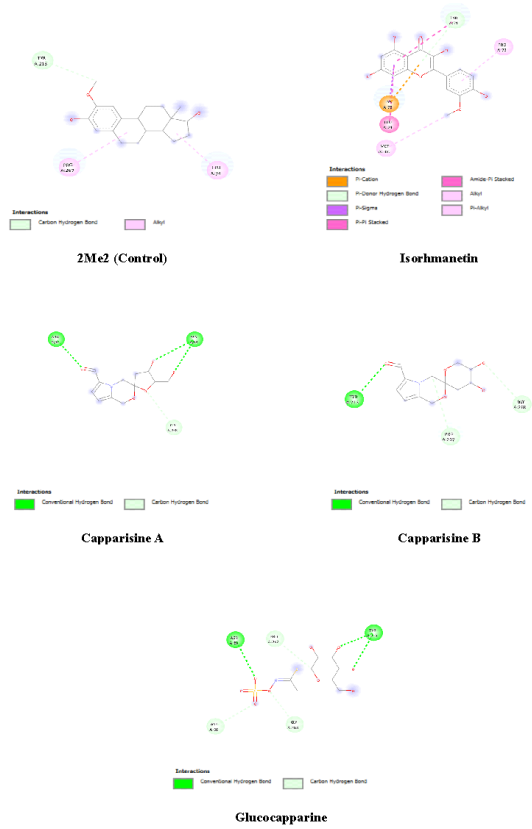

Fig 8. 2D dimensional visualization output from the molecular docking process conducted on HIF1A

AKT1 is an essential protein that affects numerous pathways associated with the inactivation of proteins that promote apoptosis, such as BAD and procaspase-9. The active form of AKT1 will lead to the phosphorylation of proteins, which affects cancer cell development, such as TSC1 and 2, that will activate mTOR once phosphorylated. Other proteins phosphorylated by AKT1 are PRAS49, YAP, p21, p27, and other proteins involved in the cell cycle and cancer development (Shariati & Bernstam, 2019). Mutation in AKT1 was investigated in 8.2% of breast cancer. The ability of a compound to inhibit this protein will interfere with several critical signalling pathways, especially PI3K/AKT.

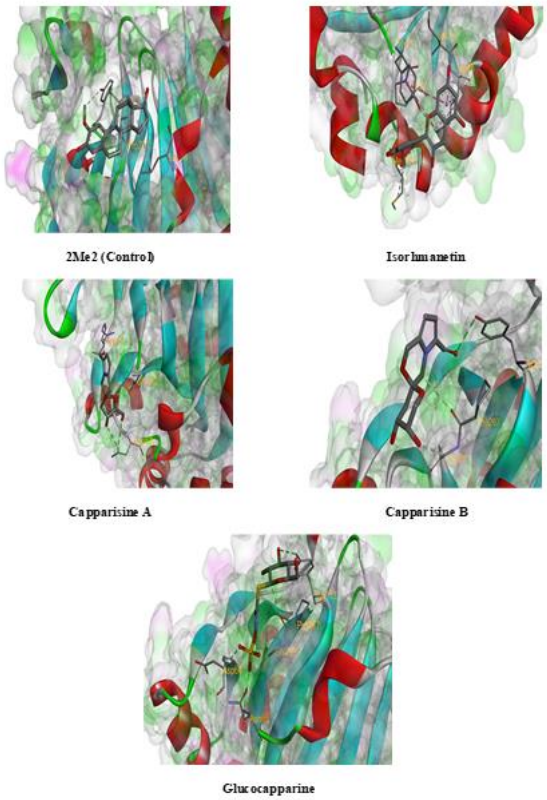

Fig 9. 3-dimensional visualization output from the molecular docking process conducted on HIF1A

Table 5. Output of Molecular Docking in HIF1A

Compounds Name	RMSD	Afinity (Kcal/Mol)	Position of Chemical Interactions		
			Hydrogen Bonds	Electrostatic Interactions	Hydrophobic Interactions
2Me2 (Control)	1.782	-4.7	TYR: 213	-	LEU: 74, PRO: 267
Isorhamnetin	1.336	-5.2	TYR: 71	LYS: 75, TYR:71, LEU: 74	LYS:75, MET:160, PRO:72
Capparisine A	1.674	-4.4	ARG: 44, ARG:68, GLY 268	-	-
Capparisine B	1.822	-4.3	TYR: 213, PRO: 267, GLY: 268	-	-
Glucocapparine	1.337	-4.7	ASN: 68, PRO:367, ASP: 66, GLY: 268, TYR: 213	-	-

The visualization shows Isorhamnetin formed a hydrogen bond to TYR 71 in HIFA. The other chemical interactions which affect binding affinity also showed in LYS 75, LEY 74, MET 160, dan PRO: 72.

Isorhamnetin also showed the highest affinity compared to each compound and the control 2Me2 in HIF1A. The affinity of the control even appeared to be lower compared to Isorhamnetin. The control 2Me2 or 2-methoxy estradiol is a new compound which shows antineoplastic activity and the ability to inhibit HIF1A. Thus, the compound that shows the ability to inhibit this protein can be considered a potential anticancer agent.

Table 6. Oral Drug-Likeness According to pkCSM Analysis Result

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Compound	Molecular Weight (Da)	LogP	H Bond Acceptor	H Bond Donor	Number of Discrepancy Towards Lipinski's Rule of 5
Isorhamnetin	316.265	2.291	7	4	0
Capparisine A	253.254	-0.3308	6	2	0
Capparisine B	253.254	-0.3308	6	2	0
Glucocapparine	333.34	-2.3277	10	5	0

The evaluation of oral drug-likeness can be conducted by considering specific factors outlined in Lipinski's Rule of Five (Ro5). A molecule that satisfies the requirements outlined in the Rule of Five (Ro5) possesses the potential to be considered a viable candidate for oral medication administration. In contrast, the chemical that violates at least two of the four requirements specified in the rule exhibits limited absorption within the gastrointestinal system, diminishing its prospects as a viable option for oral drug administration (Rokoski, 2023). The requirements outlined in the Rule of Five (Ro5) include a maximum molar mass of 500 for a chemical, a maximum of 10 hydrogen bond acceptors, a maximum of 5 hydrogen bond donors, and a maximum log P or lipophilicity value of 5 (Ivanovic et al., 2020). Considering molecular weight is crucial due to its impact on the body's absorption process, as evidenced by Fadlan et al. (2021), which found that low molecular weights facilitate and ease absorption. The parameter LogP, which indicates lipophilicity, is also a crucial factor to consider. A compound's lipophilicity is inversely proportional to its logP value. According to Syahputra et al. (2014), an optimal logP range for a medication candidate falls within the values of -0.4 to 5. The quantity of hydrogen bond acceptors and donors is a significant parameter, as a higher count of donors and acceptors of hydrogen bonds in a compound will result in a greater inclination to bind with components possessing more potent hydrogen bonding properties, as opposed to lipophilic cell membranes (Ivanovic, 2020). Based on the findings of the pkCSM Assay conducted in this study, it is observed that all the substances analyzed exhibit complete adherence to Lipinski's Rule of Five, with no discrepancies identified. All the compounds satisfy the established criteria and are anticipated to possess a favourable attribute of oral drug-likeness. According to the data, these chemicals may potentially develop in an oral dosage form, particularly for further and more in-depth studies.

The pharmacokinetic properties of the compounds are also analyzed, involving ADMET properties. Each property is supported by several parameters that are used to determine whether the pharmacokinetic property is good or vice versa. In absorption properties, several parameters are observed such as water solubility, CaCo2 permeability, % absorbed compounds in the intestine, skin permeability, as well as substrate and inhibitor properties of the compounds toward p-glycoprotein. Water solubility will support the compound to be absorbed. A compound with water solubility less than -6 log mol/l is assumed to have low water solubility (Abdullah et al., 2021). In this research, all the compounds have solubility values above -6 log mol/l. CaCo2 permeability represents the compounds' ability to permeate the gastrointestinal tract. According to Firdausy et al. (2020), a compound has a high permeability if the CaCo2 permeability is more than 0.90 Papp. In this study, the compounds showed permeability lower than 0.90 Papp.
Another parameter in absorption properties is the presentation of the absorbed compound in the intestine. A compound is considered poorly absorbed if it is lower than 30% (Abdullah et al., 2021). All the compounds show percentages above 30% except glucocapparine (0%). The possibility of a compound being the substrate of p-glycoprotein transport is also an important parameter. The compound, which is not a substrate of p-glycoprotein, can be absorbed without being expelled from the cell through this transport (Firdausy et al., 2020). Every compound except isorhamnetin is not a substrate of this transport. The parameter of skin permeability is important to be observed in case the compound is designed into a topical dosage form. A compound is considered to have poor skin permeability if the value of logKP is lower than -2.5 (Abdullah et al., 2021). In this research, the compounds show poor skin permeability with logKP lower than -2.5. A compound with a good absorption profile will also show a good distribution profile.

There are several parameters in distribution properties. The first parameter in the distribution profile is VDss (Log L/kg). The higher the value of Vdss, the higher the concentration of drugs distributed to the tissue compared to the plasma. VDss is low if it is below -0.15 and high if it is above 0.45 log L/Kg (Firdausy et al., 2020). In this research, isorhamnetin shows a high VDss value, Capparisine B shows a moderate VDss, while Capparisine A and Glucocapparine show a low VDss value. Another parameter in the distribution profile is the unbound fraction, which represents the ability of drugs to bind with plasma protein. The higher the unbounded fraction, the more effective a compound is to diffuse through the cell membrane. The unbounded fraction value is low if it is below 0.05 and considered high above 0.20 (Watanabe, 2018). All the compounds show unbounded fractions higher than 0.05. Capparisine A and B, as well as glucocapparine, even show a high value of unbounded fraction above 0.20. The ability of a compound to reach the blood-brain barrier is vital in case the compound is targeting the brain and central nervous system. A compound is considered to have the ability to pass the blood-brain if the logBB is above 0.3 (Firdausy et al., 2020). In this research, the compounds show a lower value, thus the blood-brain barrier permeability is low. The ability of the compound to pass through the central nervous system is represented by log PS, in which if the value is above 2, then the permeability of CNS is good. In this research, the log PS of the compound is below 2. Which means the CNS permeability is terrible.

Metabolism profile predicted through the ability of compounds to become the substrate or inhibitor of CYP450 enzyme isoforms. All compounds are not substrates nor

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inhibitors of cytochrome isoforms predicted in pkCSM analysis, except Isorhamnetin, which is predicted to be an inhibitor of CYP2C19. The compounds presumably metabolized in the body through another isoform of CYP450 which is not available in pkCSM prediction. Regarding excretion of the compounds, the total clearance of each compound is 0.508 log ml/min/kg for isorhamnetin, 0.432 log ml/min/kg for capparisine A, 0.407 log ml/min/kg for capparisine B, 0.372 log ml/min/kg for glucocapparine.

According to Vijay (2018)., AMES toxicity was conducted to observe the mutagenic potential of a compound. In this research, the compounds do not show the AMES toxicity potential. All the compounds are not hepatotoxic and do not lead to skin sensitization. The cardiotoxicity can be evaluated towards the possibility of the compound inhibiting hERG 1 and 2 genes (Stergiopoulus et al., 2022), and all the compounds are not the inhibitors of these genes. Maximum tolerated dose in humans, LD50 and LOAEL in rats, and other predicted pharmacokinetic toxicity can be observed in Table 7.

Table 7. Pharmacokinetic prediction According to pkCSM Analysis Result

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Pharmacokinetic Properties	Parameters	Compounds			
		Isorhamnetin	Capparisine A	Capparisine B	Glucocapparine
Absorption	Water solubility (Log mol/L)	-3	-2.069	-2.749	-1.972
	CaCo2 permeability (log Papp in 10^{-6} cm/s)	-0.003	0.66	0.627	-0.681
	Intestinal absorption (human) (% Absorbed)	76.014	80.153	78.773	0
	Skin Permeability (-2.735)	-2.735	-3.671	-3.529	-2.735
	P-glycoprotein Substrate (Yes/No)	Yes	No	No	No
	P-glycoprotein I Inhibitor (Yes/No)	No	No	No	No
	P-glykoprotein II Inhibitor (Yes/No)	No	No	No	No
Distribution	*VDss* (Log L/kg)	1.123	-0.258	0.015	-2.735
	Fraction Unbound (Fu)	0.091	0.684	0.716	0.787
	Blood Brain Barrier Permeability (Log BB)	-1.135	-0.268	-0.067	-1.483
	Central Nervous System Permeability (log PS)	-3.188	-3.491	-3.112	-3.872
Metabolism	Substrate CYP2D6 (Yes/No)	No	No	No	No
	Substrate CYP3A4 (Yes/No)	No	No	No	No
	Inhibitor CYP1A2 (Yes/No)	Yes	No	No	No
	Inhibitor CYP2C19 (Yes/No)	No	No	No	No
	Inhibitor CYP2C9 (Yes/No)	No	No	No	No
	Inhibitor CYP2D6 (Yes/No)	No	No	No	No
	Inhibitor CYP3A4 (Yes/No)	No	No	No	No
Excretion	Total Clearance (log ml/min/kg)	0.508	0.432	0.407	0.372
	Substrate OCT2 (Yes/No)	No	No	No	No
Toxicity	AMES Toxicity (Yes/No)	No	No	No	No
	Max. tolerated dose (human) (log mg/kg/day)	0.576	0.857	0.599	1.422
	hERG I Inhibitor (Yes/No)	No	No	No	No
	hERG II Inhibitor (Yes/No)	No	No	No	No
	Oral Rat Acute Toxicity (LD50 in mol/kg)	2.407	2.722	2.392	1.818
	Oral Rat Chronic Toxicity (LOAEL in log mg/kg/day)	2.499	2.266	1.925	3.265
	Hepatotoxicity (Yes/No)	No	No	No	No
	Skin Sensitization (Yes/No)	No	No	No	No
	T.Pyriformis Toxicity (log µg/L)	0.296	0.289	0.288	0.285
	Minnow Toxicity (log mM)	2.206	3.033	3.364	5.524

CONCLUSION AND RECOMMENDATION

According to bioinformatics methods, AKT1 and HIF1A were obtained as potential protein targets. In molecular docking to AKT1 protein, the docking score obtained for Isorhamnetin, Glucocapparine, Capparisine A, Capparisine B, and Ipatasertib as control were -6.2 kcal/mol, -5.3 kcal/mol, -4.9 kcal/mol, -4.9 kcal/mol, and -6.2 kcal/mol respectively. Meanwhile, the docking score for HIF1A protein for Isorhamnetin, Glucocapparine, Capparisine A, Capparisine B, and 2-methoxy estradiol as control was -5.2 kcal/mol, -4.7 kcal/mol, -4.4 kcal/mol, -4.3 kcal/mol, and -4.7 kcal/mol. The scores for each compound were like the controls in both proteins, indicating that the phytochemicals from *Capparis spinosa* that were analyzed

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have potential anti-breast cancer properties. Pharmacokinetic prediction for absorption, distribution, metabolism, excretion, and toxicity (ADMET) is also provided to help further studies and development for the compounds as anticancer drugs.

This study provided data from in silico methods regarding anti-breast cancer supported with pharmacokinetic activities for Isorhamnetin, Glucocapparine Capparisine A, and Capparisine B, which will be helpful as a reference for other advanced research in future. Further related in vitro or in silico analysis can be conducted to explore more about the chemopreventive and chemotherapy activities of *the Capparis spinosa* plant.

REFERENCE

Abdullah, S. S., Putra, P. P., Antasionasti, I., Rundengan, G., Suoth, E. J., Abdullah, R. P. I., & Abdullah, F. (2022). Analisis sifat fisikokimia, farmakokinetik dan toksikologi pada pericarpium pala (Myristica fragransa) secara artificial intelligence. Chemistry Progress, 14(2), 81-92.

Acikgoz, E., Güler, G., Camlar, M., Oktem, G., & Aktug, H. (2019). Glycogen synthase kinase-3 inhibition in glioblastoma multiforme cells induces apoptosis, cell cycle arrest and changing biomolecular structure. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 209, 150-164.

Ahmad, R., Ahmad, N., Naqvi, A. A., Shehzad, A., & Al-Ghamdi, M. S. (2017). Role of traditional Islamic and Arabic plants in cancer therapy. Journal of traditional and complementary medicine, 7(2), 195-204.

AL-Azawi, A. H., Ghaima, K. K., & Salih, H. H. (2018). Phytochemical, antibacterial and antioxidant activities of Capparis spinosa L. Cultivated in Iraq. Bioscience Research, 15(3), 2611-2618.

Alshahrani, M. Y., Alshahrani, K. M., Tasleem, M., Akeel, A., Almeleebia, T. M., Ahmad, I., ... & Saeed, M. (2021). Computational screening of natural compounds for identification of potential anticancer agents targeting MCM7 protein. Molecules, 26(19), 5878.

American Cancer Society. (2022). Breast Cancer Facts & Figures. Retrieved May 29, 2023, from cancer.org: https://www.cancer.org/research/cancer-facts-statistics/breast-cancer-facts-figures.html

Basar, M. A., Hosen, M. F., Paul, B. K., Hasan, M. R., Shamim, S. M., & Bhuyian, T. (2023). Identification of drug and protein-protein interaction network among stress and depression: A bioinformatics approach. Informatics in Medicine Unlocked, 101174.

Briones, Y. L., Young, A. T., Dayrit, F. M., De Jesus, A. J., & Rojas, N. R. L. (2021). Visualizing Phytochemical-Protein Interaction Networks: Momordica charantia and cancer. Frontiers in Bioinformatics, 1, 768886.

Chang, Y., Hawkins, B. A., Du, J. J., Groundwater, P. W., Hibbs, D. E., & Lai, F. (2023). A Guide to In Silico Drug Design. Pharmaceutics, 15(1), 49.

Coleman, N., Moyers, J. T., Harbery, A., Vivanco, I., & Yap, T. A. (2021). Clinical Development of AKT Inhibitors and Associated Predictive Biomarkers to Guide Patient Treatment in Cancer Medicine. Pharmacogenomics and personalized medicine, p. 14, 1517–1535. https://doi.org/10.2147/PGPM.S305068

Fadlan, A., Warsito, T., & Sarmoko, S. (2022). Studi In Silico Potensi Antikanker Senyawa Kaempferida. ALCHEMY: Journal of Chemistry, 10(1), 14-21.

Firdausy, A. F., Mutiah, R., & Rahmawati, E. K. (2020). Predicting pharmacokinetic profiles of sunflower's (Helianthus L.) active compounds using in silico approach. Journal of Islamic Medicine, 4(1), 1-7.

Hameed, A. T., Zaidan, D. H., & Dawd, S. M. (2021). The Phytochemical Constituent Of Capparis Spinosa L. And Phenolic Activity On Pathogenic Bacteria And Blood Parameters.

Hameed, A. T., Zaidan, D. H., & Dawd, S. M. (2021). The Phytochemical Constituent Of Capparis Spinosa L. And Phenolic Activity On Pathogenic Bacteria And Blood Parameters.

Hameed, A. T., Zaidan, D. H., & Dawd, S. M. (2021). The Phytochemical Constituent Of Capparis Spinosa L. And Phenolic Activity On Pathogenic Bacteria And Blood Parameters.

He, Y., Sun, M. M., Zhang, G. G., Yang, J., Chen, K. S., Xu, W. W., & Li, B. (2021). Targeting PI3K/Akt signal transduction for cancer therapy. Signal transduction and targeted therapy, 6(1), 425.

Hinz, N., & Jücker, M. (2019). Distinct functions of AKT isoforms in breast cancer: a comprehensive review. Cell Communication and Signaling, 17, 1-29.

Hoxhaj, G., & Manning, B. D. (2020). The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. Nature Reviews Cancer, 20(2), 74–88.

Huang, H., Zhang, G., Zhou, Y., Lin, C., Chen, S., Lin, Y., ... & Huang, Z. (2018). Reverse screening methods to search for the protein targets of chemopreventive compounds. Frontiers in chemistry, 138.

Huang, M., Lu, J. J., & Ding, J. (2021). Natural products in cancer therapy: Past, present and future. Natural products and bioprospecting, 11, 5-13.

Infantino, V., Santarsiero, A., Convertini, P., Todisco, S., & Iacobazzi, V. (2021). Cancer cell metabolism in hypoxia: Role of HIF-1 as key regulator and therapeutic target. International journal of molecular sciences, 22(11), 5703.

Ivanović, V., Rančić, M., Arsić, B., & Pavlović, A. (2020). Lipinski's rule of five, famous extensions and famous exceptions. Popular Scientific Article, 3(1), 171-177.

Janku, F., Yap, T. A., & Meric-Bernstam, F. (2018). Targeting the PI3K pathway in cancer: are we making headway?. Nature reviews Clinical oncology, 15(5), 273-291.

Jeknic, S. (2022). Development of a Live-Cell HIF-1α Reporter and Characterization of Single-Cell HIF-1α Signaling. Stanford University.

Ji, X., Lu, Y., Tian, H., Meng, X., Wei, M., & Cho, W. C. (2019). Chemoresistance mechanisms of breast cancer

ISSN 2961-7758

and their countermeasures. Biomedicine & Pharmacotherapy, 114, 108800.

Jing, X., Yang, F., Shao, C., Wei, K., Xie, M., Shen, H., & Shu, Y. (2019). Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Molecular cancer, pp. 18, 1–15.

Kaboli, P. J., Imani, S., Jomhori, M., & Ling, K. H. (2021). Chemoresistance in breast cancer: PI3K/Akt pathway inhibitors vs the current chemotherapy. American Journal of Cancer Research, 11(10), 5155.

Kaboli, P. J., Rahmat, A., Ismail, P., & Ling, K. H. (2015). MicroRNA-based therapy and breast cancer: A comprehensive review of novel therapeutic strategies from diagnosis to treatment. Pharmacological research, pp. 97, 104–121.

Lee, Y.-R., Chen, M., and Pandolfi, P. P. (2018). The functions and regulation of the PTEN tumor suppressor: new modes and prospects. Nat. Rev. Mol. Cell Biol. 19 (9), 547–562. doi:10.1038/s41580-018-0015-0

Luo, S., Jiang, Y., Zheng, A., Zhao, Y., Wu, X., Li, M., ... & Shen, J. (2022). Targeting hypoxia-inducible factors for breast cancer therapy: A narrative review. Frontiers in Pharmacology, 13.

Martorana, F., Motta, G., Pavone, G., Motta, L., Stella, S., Vitale, S. R., ... & Vigneri, P. (2021). AKT inhibitors: new weapons in the fight against breast cancer?. Frontiers in Pharmacology, 12, 662232.

Martorana, F., Motta, G., Pavone, G., Motta, L., Stella, S., Vitale, S. R., ... & Vigneri, P. (2021). AKT inhibitors: new weapons in the fight against breast cancer?. Frontiers in Pharmacology, 12, 662232.

Mendoza, S. V., Genetos, D. C., & Yellowley, C. E. (2023). Hypoxia-Inducible Factor-2α Signaling in the Skeletal System. JBMR plus, 7(4), e10733.

Miricescu, D., Totan, A., Stanescu-Spinu, I. I., Badoiu, S. C., Stefani, C., & Greabu, M. (2020). PI3K/AKT/mTOR signaling pathway in breast cancer: from molecular landscape to clinical aspects. International journal of molecular sciences, 22(1), 173.

Mohan, H. (2018). Textbook of pathology. Jaypee Brothers Medical Publishers.

Najmi, A., Javed, S. A., Al Bratty, M., & Alhazmi, H. A. (2022). Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. Molecules, 27(2), 349.

Nitulescu, G. M., Van De Venter, M., Nitulescu, G., Ungurianu, A., Juzenas, P., Peng, Q., ... & Margina, D. (2018). The Akt pathway in oncology therapy and beyond. International journal of oncology, 53(6), 2319-2331.

Park, H., & Ma, C. (2018). Updates on AKT Inhibition in Estrogen Receptor Positive Breast Cancer. Medical Research Archives, 6(1).

Peacock, D. L. (2013). Identification of Novel HIF1A Target Genes That Regulate Tumor Progression and Metastasis. The University of Tennessee Health Science Center.

Peiró, C. H. F., Encina, J. A., Perez, M. M., Aquino, G. S., Veiga, G. L., Fonseca, F., & Alves, B. C. (2019). The role of hypoxia-induced factor 1α in breast cancer. Journal of Cancer Metastasis and Treatment, 5, 49.

Program Department of Defense Breast Cancer Research. (2021, - -). The Breast Cancer Landscape. Retrieved May 29, 2023, from https://cdmrp.health.mil: https://cdmrp.health.mil/bcrp/pdfs/Breast%20Cancer%20Landscape2021.pdf

Roskoski Jr, R. (2023). Rule of five violations among the FDA-approved small molecule protein kinase inhibitors. Pharmacological Research, 106774.

Shariati, M., & Meric-Bernstam, F. (2019). Targeting AKT for cancer therapy. Expert opinion on investigational drugs, 28(11), 977-988.

Song, M., Bode, A. M., Dong, Z., & Lee, M. H. (2019). AKT as a Therapeutic Target for CancerChallenging Cancer Therapy by Targeting AKT. Cancer Research, 79(6), 1019-103

ISSN 2961-7758