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Original Article

Molecular Docking Estrogen Receptor Alpha Antagonist and P53-MDM2 Inhibitor, ADMET Prediction of Alkaloid Compound from *Mitragyna speciosa* for Breast Cancer Therapy

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ABSTRACT

Introduction: Breast cancer is one of the major universal health problems affecting more than two million cases per year. Estrogen receptor alpha (ERα) and P53 are common targets for the treatment of breast cancer and are primarily involved in cell proliferation. The function of p53 protein is regulated by direct binding to MDM2 protein. Therefore, inhibition of p53-MDM2 interaction leads to reactivating p53 activity. Alkaloid compounds generally have potential anticancer effect. Alkaloid compound from *Mitragyna speciosa* have the potential for anticancer. **Methods:** The method used is molecular docking with AutoDockTools 1.5.6 program. Predict the properties of physicochemical, pharmacokinetic, and toxicity prediction tests (ADMET) using pkCSM. **Results:** The results showed that speciophylline, corynoxine A, and corynoxine B have the best values in free binding energy (ΔG) for estrogen receptor (ERα) alpha receptor. Meanwhile, mitraphylline, mitrafoline, and corynoxine B have the best values for protein P53. Predict ADMET using the pkCSM, the alkaloid compound has strong lipophilicity and good permeability so it predicts the ability to penetrate intestinal cell membranes and the skin membrane. Spesiofilin, mitraphylline, and mitrafolin are not expected hepatotoxic. **Conclusion:** Speciophylline and mitraphylline have potential as anticancer drugs through the inhibitory of estrogen receptor alpha and MDM2 reseptor.
Key words: ADMET, Alkaloid, Breast Cancer, Docking, *Mitragyna speciosa*.

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INTRODUCTION

Cancer is a disease with uncontrolled and abnormal cell growth. Breast cancer is a disease with a high prevalence and has become a burden for health worldwide. According to GLOBOCAN (Global Burden of Cancer) data in 2020 was new cases because breast cancer is the first order in the world with 2,261,419 cases (11.7%), then second place was lung cancer with 2,206,771 cases (11.4%).[1]

Factors that influence the prognosis of breast cancer include hormonal factors (such as estrogen receptors (ER) and progesterone receptors (PR)) and growth factors (such as Epidermal Growth Factor Receptor (EGFR)). Therefore, some treatments for breast cancer target receptors such as tamoxifen (an antiestrogen) and trastuzumab (anti-Human Epidermal Growth Factor Receptor-2).[2] Another factor affecting the prognosis of breast cancer is the accumulation of p53. p53 is a tumor suppressor protein that influences cell cycle control, DNA repair, and induces programmed death (apoptosis) when cells are damaged.[3] Inactivation of p53 is common in breast cancer due to p53 mutations and increased levels of MDM2 (murine double minute 2) that can be correlated with poor clinical outcomes.[4,5]

MDM2 acts as a negative regulator of p53 that can inhibit p53 transcriptional activity.[6] The function of the p53 protein is regulated by direct binding to the MDM2 protein. Therefore, inhibition of p53-MDM2 interaction leads to reactivating p53 activity. The relationship between MDM2 and estrogen has implications for p53 inhibition. Two-thirds of breast cancer growth depends on estrogen.[7] Estrogen induces transcription of target genes to activate cell proliferation by inducing BCL-2 (anti-apoptotic) gene expression thereby inhibiting programmed cell death. Estrogen can inhibit p53 transcriptional activity and increase mdm2 expression. MDM2 can improve ERA function.[8,9]

Natural products and medicinal plants have been widely proposed as agents for the prevention and treatment of cancer.[10] Compounds obtained from nature that have the potential as anticancer can come from the alkaloid group, such as vinblastine and vincristine.[11] Plant materials that contain alkaloid compounds and have the potential as anticancer can be derived from kratom leaf (*Mitragyna speciosa*). Extracts, fractions, and alkaloids compound from *Mitragyna speciosa* have the potential for anticancer neuroblastoma, leukemia, colon cancer, and breast cancer.[12-14]

Mitragyna spesiosa leaves contain an alkaloid compound known as the dominantly active compound (mitragynine) and 54 other types of alkaloids.[15] Mitragynine shows high cytotoxic and antiproliferative activity against colon cancer and erythroleukemia.[13] The potential content of other alkaloid compounds in an anticancer activity needs further investigation, especially for breast cancer. The study of the alkaloid compound *Mitragyna spesiosa*, which inhibits estrogen receptor alpha (ERα) and MDM2 protein, takes time. Therefore, a molecular approach such as molecular docking is needed to test the content of natural compounds for breast

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cancer anticancer potential as a preliminary study to determine the active alkaloid content of the *Mitragyna spesiosa*. In silico prediction of pharmacokinetic and toxicity properties are needed to show the chemical compounds that are good for the absorption, distribution, metabolism, excretion, and toxicity of a compound that will later become a drug candidate.[16]

METHODS

Tools and materials

The hardware used was a laptop Legion 5 Pro 16AC6H with 16.0 gigabyte RAM (Random Access Memory) specifications, AMD Ryzen 7 5800H Processor, Radeon graphics 3.20 GHz, NVIDIA GeForce RTX 3060 Graphic Card, Microsoft® Windows® 10 Pro operating system. The software used is Avogadro for energy minimization and Swiss PDB viewer for protein optimization. Molecular docking using Autodock 4.2.6, and for visualization of protein-ligand interactions using the Discovery studio visualizer and PyMOL. ADMET predictions use the pkCSM website. The material used in this study was a 3D structure of the alkaloid compound contained in *Mitragyna speciosa*. The molecular structures of the tested compounds were mitragynine, paynantheine, speciofoline, mitraphylline, speciophylline, corynoxineB, isomitraphylline, mitraciliatine, corynoxineA, corynantheidine, isorhynchophylline, mitrafoline, ajmalicine, speciociliatine, isospeciofoline, mitragynaline, 7-hydroxymitragynine, rhynchophylline, and speciogynine. [17]

Protein and ligand preparation

The protein crystal structure of the Human Estrogen Receptor Alpha Ligand-Binding Domain and the Humanized Xenopus MDM2 were obtained from the Protein Data Bank with PDB ID 3ERT (Estrogen Receptor), 4LWU (MDM2 Receptor). The target protein was prepared by removing water and adding hydrogen using the Discovery Studio Visualizer. Then the protein structure is optimized using Swiss PDB viewer with the force field setting used as GROMOS96, and saved in PDB file format. Total 19 alkaloid compounds contained in *Mitragyna speciosa* Korth will be test in this study. These compounds were obtained from PubChem and positive control compounds used native ligand compounds for each protein, namely 4-hydroxytamoxifen (Estrogen Receptors) and RO5499252 (MDM2 Receptors). This compound was prepared by adding hydrogen followed by energy minimization using Avogadro software with the force field setting used MMFF94.

Molecular docking

Molecular docking of the *Mitragyna speciosa* compound to two PDB ID proteins, such us 3ERT (estrogen receptor) and 4LWU (MDM2 receptor) was performed using Autodock 4.2.6 software. The first thing it does is upload the protein and ligand into the autodock, detection, and determination of the torque for the Gasteiger and Kollman partial loads are automatically added to the test compound. Then in the settings of the grid box, the results of the validation method are used where the size of the grid box used is 40 x 40 x 40 with coordinates (X= 30,323, Y= -1,690, Z= 23,716) for the estrogen receptor protein and (X= -11,672, Y= 21.331, Z= -5.754) for MDM2 Receptors and the default distance used is 0.375 Å. The running docking setting uses the Lamarckian Genetic Algorithm with a population size of 150 and the maximum number of evaluations is 2,500,000 for every 100 runs. Evaluation of the docking results is the best conformation marked by the binding energy score (ΔG) with the lowest inhibition constant (Ki) as well as functional essential amino acid interactions that are detected to play a role in docking interactions by Discovery Studio Visualizer.

ADMET predictions

Predictions from ADMET use web-based software, namely pKCSM (https://biosig.lab.uq.edu.au/pkcsm/). pKCSM is used to predict the pharmacokinetic properties and toxicity of chemical compounds. pKCSM is used to reduce research costs and increase the potential for highly accurate and fast predictions.[18] Total 19 compounds showing the results of molecular docking were screened by ADMET by changing the PDB format to SMILES using Open Babel software and then uploaded to pKCSM.

RESULTS AND DISCUSSION

Molecular docking

Estrogen receptors are classified into two, such us ERβ and ERα. These receptors are encoded by different genes located on different chromosomes. The human ERα gene located on chromosome 6 while

Table 1: The docking results of alkaloid compounds and native ligand against 3ERT.

Compound	RMSD	ΔG (Kcal/mol)	Ki
Ligand Native	1.195 A	-12,36	867.83 pM
Speciophylline	40.035 A	-9,39	130.56 nM
CorynoxineA	39.750 A	-9,22	174.37 nM
CorynoxineB	39.223 A	-9,20	180.35 nM
Isomitraphylline	38.437 A	-8,88	312.26 nM
Mitraciliatine	38.275 A	-8,86	317.64 nM
Corynantheidine	38.250 A	-8,82	345.44 nM
Isorhynchophylline	40.262 A	-8,62	480.60 nM
Ajmalicine	36.945 A	-8,57	526.73 nM
Speciociliatine	35.700 A	-8,54	552.39 nM
7-Hydroxymitragynine	36.950 A	-8,50	587.74 nM
Isospeciofoline	38.987 A	-8,21	958.75 nM
Mitrafoline	37.364 A	-7,97	1.43 uM
Rhynchophylline	39.612 A	-7,96	1.47 uM
Speciogynine	36.812 A	-7,96	1.46 uM
Mitragynine	35.210 A	-7,90	1.62 uM
Paynantheine	35.820 A	-7,85	1.75 uM
Speciofoline	40.035 A	-7,60	130.56 nM
Mitraphylline	41.019 A	-6,86	9.29 uM
Mitragynaline	36.827 A	-6,84	9.62 uM

Table 2: The docking results of alkaloid compounds and native ligand against MDM2.

Compound	RMSD	ΔG (Kcal/mol)	Ki
Ligand Native	2.470 A	-10,54	18.73 nM
Mitraphylline	20.962 A	-8,01	1.34 uM
Mitrafoline	21.297 A	-7,50	3.17 uM
CorynoxineB	21.200 A	-7,42	3.61 uM
Isomitraphylline	21.429 A	-7,39	3.83 uM
Isospeciofoline	20.990 A	-7,36	4.04 uM
Speciofoline	22.292 A	-7,21	5.16 uM
Ajmalicine	19.259 A	-7,17	5.56 uM
Paynantheine	19.037 A	-7,06	6.66 uM
Speciophylline	22.109 A	-6,99	7.52 uM
Speciogynine	21.869 A	-6,92	8.47 uM
Corynantheidine	22.205 A	-6,88	9.13 uM
Isorhynchophylline	20.567 A	-6,80	10.33 uM
CorynoxineA	19.884 A	-6,70	12.29 uM
Mitragynine	21.908 A	-6,68	12.79 uM
Speciociliatine	18.609 A	-6,64	13.57 uM
Mitragynaline	20.422 A	-6,57	15.16 uM
Mitraciliatine	19.635 A	-6,31	23.89 uM
7_Hydroxymitragynine	19.141 A	-5,99	40.92 uM
Rhynchophylline	18.628 A	-5,97	42.32 uM

Table 3: Parameters of ADMET of Speciophylline, Corynoxine, Mitraphylline, and Mitrafoline.

ADMET	Parameters	Mitraphylline	Mitrafoline	CorynoxineB	Speciophylline	CorynoxineA
Absorption	Intestinal absorption (%)	95,569	94,950	94,024	95,569	94,024
	Skin permeability (log Kp)	-2,893	-2,739	-2,776	-2,893	-2,776
Distribution	VDss (human) (log L/kg)	0,934	0,892	0,698	0,934	0,698
	BBB permeability (log BB)	-0,063	-1,25	0,094	-0,063	0,094
	CNS permeability (log PS)	-2,274	-2,592	-2,403	-2,274	-2,043
Metabolism	CYP3A4 substrate (Yes/No)	Yes	No	Yes	Yes	Yes
Excretion	Total Clearance (log ml/min/kg)	0,903	0,835	0,868	0,903	1,868
	Renal OCT2 substrate (Yes/No)	No	No	No	No	No
Toxicity	AMES toxicity (Yes/No)	No	No	No	No	No
	Max. tolerated dose (log mg/kg/day)	-1,244	-0,613	-0,741	-1,244	0,259

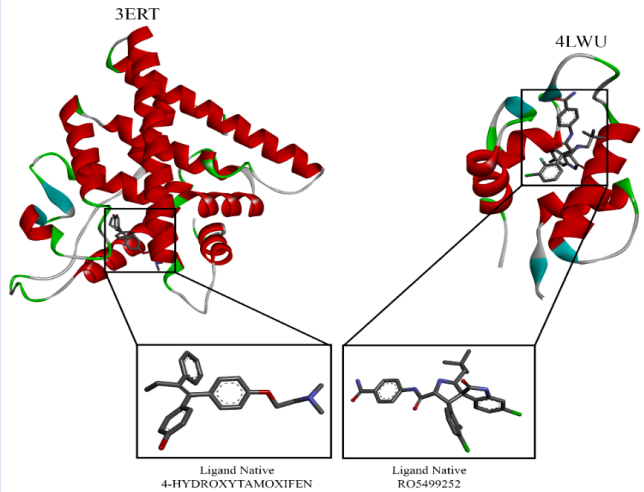

Figure 1: Visualization of ligand native and targeted protein.

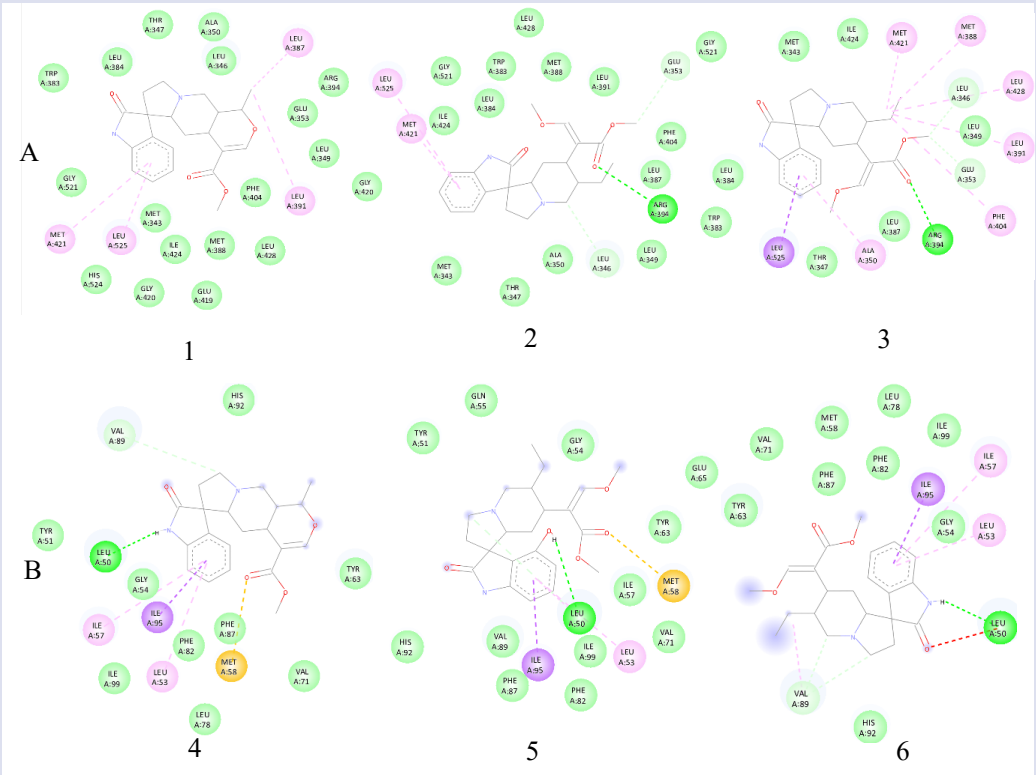

Figure 2: The binding visualization of A) Estrogen receptor alpha and B) MDM2 targeting 1) Speciophylline, 2) CorynoxineA, 3) CorynoxineB, 4) Mitraphylline, 5) Mitrafoline, 6) CorynoxineB. Note: green = hydrogen, light green = van der Waals, pink = pi-alkyl, purple = pi-sigma, and orange = pi-cation.

the ERβ gene is on chromosome 14. These receptors drive genes involved in cell proliferation. Both receptors have distinct and unique roles in the immune, skeletal, cardiovascular and central nervous systems and are also involved in morphogenesis.[19] The results of the molecular binding of the *Mitragyna speciosa* plant alkaloid compound to 3ERT protein (estrogen receptor) are shown in table 1.

The highest docking results was seen based on the strongest of free bond energy value, namely the native ligand (4-hydroxytamoxifen) with a value of -12.36 kcal/mol, followed by the speciophylline compound with a free bond energy value of -9.39 kcal/mol, and corynoxine A is -9.22 kcal/mol. The highest compound was the positive control compound, namely 4-hydroxytamoxifen (OHT). OHT is a compound used in clinical use in cancer therapy. The effectiveness therapeutic of TAM (tamoxifen) in the treatment of cancer is hormone dependent. Preventing breast cancer in high-risk women is predicted to arise primarily from its ability to compete with estrogen for binding to ER. TAM and its active metabolites can actively induce programmed cell death through different ER-dependent and ER-independent pathways.[20]

The speciophylline compound has not been studied as active against the estrogen receptor. However, speciophylline from the *Uncaria tomentosa* plant has a broad range of activities against malignant melanoma (SKMEL), epidermoid carcinoma (KB), ductal carcinoma (BT-549), and ovarian carcinoma (SK-OV-3).[21] The alkaloids compound of *U. tomentosa* consisting uncarine, speciophylline, pteropodine, mitraphylline, isomitraphylline, and isopteropodine. These alkaloid compounds increased the proapoptotic response of HL-60 promyelocytic leukemia cells.[22] Speciophylline showed significant and dose-dependent cytotoxic activity against T24 and RT4 human bladder cancer cells with IC_{50} of 164.13 and 137.23 μg/mL, respectively.[21]

The studies on the therapeutic effects of Cory (Corynoxine) on cancer are limited. Cory had been increasing the chemosensitivity of liver carcinoma cells that are resistant to doxorubicin and function as a reversal agent for multidrug resistance in liver carcinoma.[23] However, it remains unclear whether Cory has any biological effect on pancreatic cancer. Cory induces G2/M phase termination. The cycles of cell arrest induced in response to DNA damage, we further investigated whether Cory could induce DNA damage in pancreatic cancer cells.[24]

MDM consists of the E3 ligase MDM2 and its close homologue MDM4 (also known as MDMX). The MDM family is located in epithelial cells, lining the lumen of the milk ducts, but not visible in the surrounding stroma.[25] The MDM family is best characterized for dynamic negative regulation of the major tumor suppressor p53. MDM2 also has p53-independent functions, in cell cycle control, differentiation, cell fate determination, DNA repair, basal transcription, and other processes.[26] The results of the molecular binding of the *Mitragyna speciosa* Korth plant alkaloid compound to the 4LWU protein (MDM2 receptor) are shown in table 2.

The corynoxine A and corynoxine B compounds show the amino acid residue ARG 394, where the formation of hydrogen bonds with Arg394 is very important for binding ERα.[27] Whereas in the compounds Mitraphylline, Mitrafoline, and Corynoxine B LEU 50 is an amino acid with many types of hydrogen bonding that plays a role in targeting MDM2.[28] This shown in figure 2.

The tumor suppressor p53 is a potent transcription factor that plays a central role in maintaining the integrity of the cell genome. p53 is controlled by a negative regulator, MDM2. In about 50% of human cancers, p53 remains wild-type but its function is impaired by other mechanisms, such as overexpression of MDM2.[29,30] The Mitraphylline has antiproliferative and cytotoxic effects that have been tested in human Ewing sarcoma MHH-ES-1 and MT-3 breast cancer cell lines using cyclophosphamide and vincristine as controls. Micromolar concentrations of mitraphylline (5 μM to μ40 M) could inhibit the growth of both cell lines. The IC_{50} values of mitraphylline were 17.15 μM for MHH-ES-1 and 11.80 μM for MT-3 for 30 hours, which were lower IC_{50} than those obtained by the reference compounds. These actions suggest that the pentacyclic oxindole alkaloid mitraphylline may be a promising new agent in the treatment of human sarcoma and breast cancer.[31]

Mitrafoline does not yet have research data proving its activity as an anti-breast cancer. This compound contained <1% of the alkaloid fraction of *Mitragyna speciosa* leaves.[17] This plant alkaloid fraction has anticancer activity in nasopharyngeal cancer with an IC_{50} value of 32.16 ± 0.94 μg/ml.[32] Mitrafoline is a compound belonging to the indole alkaloid group. Indole alkaloids and their derivatives have been widely used therapeutically in clinical practice to treat various types of cancer, such as malignant lymphoma, lung cancer, acute leukemia, and breast cancer. Indole alkaloids can target cancer autophagy processes, including the MAPK, PI3K/Akt/mTOR, Beclin-1, and ROS signaling pathways.[33]

ADMET predictions

The ADMET prediction of the three compounds with the highest bond energy values for each receptor using pKCSM can be seen in table 3. The absorption shows that the best compounds have high permeability or the ability to penetrate Caco-2 cells and have good absorption in the human intestine. Therefore, all compounds can be absorbed through the human digestive tract, namely the intestines. These compounds also have a high permeability value on the skin. This result indicates compounds can make in topical preparations. The nature of the distribution shows that compounds have high VDss values. This compounds can be widely distributed outside the network. The ability to penetrate the blood-brain barrier (BBB) Speciophylline, Corynoxine A and Corynoxine B have a good ability to penetrate the BBB. Mitraphylline and Mitrafoline compounds have a poor ability to penetrate the BBB. All compounds have low values for the central nervous system. They are not suitable to be targeted as drug candidates that work on the central nervous system.

Metabolism characteristics for Speciophylline, Corynoxine A, Corynoxine B, and Mitraphylline except for mitrafoline predicted as a substrate of the CYP3A4 enzyme so that it can be metabolized by the main cytochrome CYP3A4. Mitrafoline is predicted to be well metabolized by cytochrome P450 and easily excreted. The excretion of Corynoxine A shows the greatest total clearance value. Corynoxine A has a higher excretion rate than other compounds. All of the above compounds do not show renal OCT2 substrates indicating that all compounds do not cause toxic effects when consumed togethers with renal OCT2 inhibitors. Speciophylline, Mitraphylline, and Mitrafoline are predicted to be non-toxic to the liver (hepatotoxic), while corynoxine A and cornoxine B have hepatotoxic potential.

CONCLUSION

Speciophylline and mitraphylline have potential as anticancer drugs through the inhibitory of estrogen receptor alpha and MDM2 reseptor. The best anticancer alkaloid compounds from *Mitragyna speciosa* have good pharmacokinetic profiles and they are safe. Further studies of alkaloid compounds are needed to elucidate their potential pharmacological activities.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest in this research.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49.
2. Ray A. Cancer Prevention Role of Selected Dietary Factors. Indian J CA. 2005;2(3):15-24.
3. Yamashita H, Toyama T, Nishio M, Ando Y, Hamaguchi M, Zhang Z, *et al.* p53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. Breast Ca Res. 2006;8(4):R48.
4. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70.
5. Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W. Mono-versus polyubiquitination: differential control of p53 fate by Mdm2. Science. 2003;302(5652):1972-5.
6. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, *et al.* Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science. 1996;274(5289):948-53.
7. Martin M. Molecular biology of breast cancer. Clin Transl Oncol. 2006;8(1):7-14.
8. Sayeed A, Konduri SD, Liu W, Bansal S, Li F, Das GM. Estrogen receptor alpha inhibits p53-mediated transcriptional repression: implications for the regulation of apoptosis. Cancer Res. 2007;67(16):7746-55.
9. Kinyamu HK, Archer TK. Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. Mol Cell Biol. 2003;23(16):5867-81.
10. Cragg GM, Pezzuto JM. Natural Products as a Vital Source for the Discovery of Cancer Chemotherapeutic and Chemopreventive Agents. Med Princ Pract. 2016;25(2):41-59.
11. Harvey AL. Medicines from nature: are natural products still relevant to drug discovery. Trends Pharmacol Sci. 1999;20(5):196-8.
12. Saidin NA, Cytotoxicity of extract of Malaysian *Mitragyna speciosa* Korth and its dominant alkaloid mitragynine. [thesis]. London: Imperial College London; 2008.
13. Goh TB, Yian KR, Mordi MN, Mansor SM. Antioxidant value and antiproliferative Efficacy of Mitragynine and a Silane Reduced Analogue. Asian Pac J Cancer Prev. 2014;15(14):5659-65.
14. Suryandari P, Ardiningsih P, Widiyantoro A. Cytotoxic Compounds from the Dichloromethane Fraction of Kratom Leaves (*Mitragyna speciosa* Korth.) Against T47D Breast Cancer Cells. J Kimia Khatulistiwa. 2018;7(2):1-7.
15. Flores-Bocanegra L, Raja HA, Graf TN, Augustinović M, Wallace ED, Hematian S. The Chemistry of Kratom [*Mitragyna speciosa*]: Updated Characterization Data and Methods to Elucidate Indole and Oxindole Alkaloids. J Nat Prod. 2020;83(7):2165-77.
16. Nerdy N, Margata L, Meliala L, Purba JS, Sembiring BM, Ginting S, *et al.* In Silico Evaluation of the Physicochemical, Pharmacokinetics, and Toxicity Profiles of Sesquiterpene Lactones of South African Leaf (*Vernonia amygdalina* Delile). Trop J Nat Prod Res. 2021;5(10):1835-40.
17. Hassan Z, Muzaimi M, Navaratnam V, Yusoff NHM, Suhaimi FW, Vadivelu R, *et al.* From Kratom to mitragynine and its derivatives: Physiological and behavioural effects related to use, abuse, and addiction. Neurosci Biobehav Rev. 2013;37(2):138-51.
18. Pires DE, Blundell TL, Ascher DB. pkCSM : predicting small-molecule pharmacokinetic properties using graph-based signatures (Theory-How to Enterpret pkCSM Result). pkCSM. 2015;58(9):4066-72.
19. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, *et al.* Estrogen receptors: how do they signal and what are their targets. Physiological Rev. 2007;87(3):905-31.
20. Obrero M, Yu DV, Shapiro DJ. Estrogen receptor-dependent and estrogen receptor-independent pathways for tamoxifen and 4-hydroxytamoxifen-induced programmed cell death. J Biol Chem. 2002;277(47):45695-703.
21. Kaiser S, Dietrich F, Resende PE, De VSG, Moraes RC, Morrone FB, *et al.* Cats claw oxindole alkaloid isomerization induced by cell incubation and cytotoxic activity against T24 and RT4 human bladder cancer cell lines. Planta Med. 2013;79(15):1413-20.
22. Pilarski R, Carmen MGD, Pedro MGL, Soldevila G, Olejnik A, Grajek W, *et al.* Enhanced proapoptotic response of the promyelocytic leukemia HL-60 cells treated with an *Uncaria tomentosa* alkaloid preparation. J Herb Med. 2013;3(1):149-56.
23. Yang L, Du C, Wu L, Yu J, An X, Yu W, *et al.* Cytokine-induced killer cells modulates resistance to cisplatin in the A549/DDP cell line. J Cancer. 2017;8(16):3287-95.
24. Chenmei W, Qingqing R, Zhaofeng L, Xiang Z, Xuezhi Y. Corynoxine exerts inhibitory effects on pancreatic cancer by inducing endoplasmic reticulum stress-mediated apoptosis via ROS-p38 pathway. 2020;11(6):826-35.
25. Haupt S, Mitchell C, Corneille V, Shortt J, Fox S, Pandolfi PP, *et al.* Loss of PML cooperates with mutant p53 to drive more aggressive cancers in a gender-dependent manner. Cell Cycle. 2013;12(11):1722-31.
26. Manfredi JJ. The Mdm2–p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev. 2010;24(15):1580-9.
27. Muchtaridi M, Syahidah HN, Subarnas A, Yusuf M, Bryant SD, Langer T. Molecular Docking and 3D-Pharmacophore Modeling to Study the Interactions of Chalcone Derivatives with Estrogen Receptor Alpha. Pharmaceuticals. 2017;10(4):81.
28. Tabti K, Sbai A, Elmchichi L, Maghat H, Lakhlifi T, Bouachrine M. Computational investigation of pyrrolidin derivatives as novel GPX4/MDM2–p53 inhibitors using 2D/3D-QSAR, Molecular docking, Molecular dynamics simulations and MM-GBSA free energy. Res Square. 2022;1-23.
29. Vassilev LT. MDM2 inhibitors for cancer therapy. Trends Mol Med. 2007;13(1):23-31.
30. Wu X, Bayle JH, Olson D, Levine AJ. The p53-mdm-2 autoregulatory feedl ack loop. 1993;53(7A):1126-32.
31. García GD, García PE, Saenz RT, Fernandez AA, De LPR. Cytotoxic effect of the pentacyclic oxindole alkaloid mitraphylline isolated from *Uncaria tomentosa* bark on human ewing's sarcoma and breast cancer cell lines. Planta Medica. 2010;76(2):133-6.
32. Domnic G, Jeng-Yeou CN, Abdul RSF, Ramanathan S, Lo KW, Singh D, *et al.* Combinations of indole based alkaloids from *Mitragyna speciosa* (Kratom) and cisplatin inhibit cell proliferation and migration of nasopharyngeal carcinoma cell lines. J Ethnopharmacol. 2021;279(114391):1-10.
33. Luo ML, Huang W, Zhu HP, Peng C, Zhao Q, Han B. Advances in indole-containing alkaloids as potential anticancer agents by regulating autophagy. Biomed Pharmacother. 2022;149:112827.

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