International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Special Issue 1, 2025

Original Article

IDENTIFICATION OF LC-MS/MS AND DOCKING ANALYSIS OF TOPOISOMERASE IIA INHIBITION FROM KRATOM LEAVES *(MITRAGYNA SPECIOSA)* AS POTENTIAL ANTICANCER AGENTS

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Received: 04 Oct 2024, Revised and Accepted: 20 Nov 2024

ABSTRACT

Objective: This study aimed to determine the metabolite content of kratom leaves using LC-MS/MS and their activity to inhibit topoisomerase IIα were predicted using molecular docking.

Methods: Chemical profile based on LC-MS/MS on an ethanol extract of kratom leaves sub-extracts obtained by partitioning with n-hexane and ethyl acetate solvents. The chemical profile was predicted for its anticancer activity in inhibiting topoisomerase IIα using Autodock Vina.

Results: The LC-MS/MS results showed 25 compounds identified from the extracts and sub-extracts. The three compounds with the highest affinity for inhibiting topoisomerase IIα were rutin, 5(S)-5-carboxystrictosidine, procyanidin with energy binding scores (ΔG) of 10.78, 10.74, and 10.10 kcal/mol, respectively.

Conclusion: The findings indicated the fact that the alkaloid group compounds were the dominant compounds found in kratom leaves and had a strong potential to inhibit topoisomerase IIα.

Keywords: Alkaloid, Anticancer, Chemical profile, Docking, Kratom, LC-MS/MS, Topoisomerase

DOI: https://dx.doi.org/10.22159/ijap.2025.v17s1.18 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Cancer is the leading global cause of mortality, resulting in a reduction in the average length of life of individuals [1]. In 2022, there were about 20 million new instances of cancer and 9.7 million cancer-related deaths. According to estimates, one in five men and women will get cancer at some point in their lives, while one in nine men and one in twelve women will pass away from it. In terms of both cases and fatalities, breast cancer and lung cancer were the most common cancers in women and men, respectively [2]. Cancer is a pathological condition characterized by the proliferation of cells in an improper and unregulated manner. The risk factors for cancer include age, exposure to ultraviolet radiation, family history, genetic vulnerability, low healthy lifestyle in terms of nutrition and movement that raise the risk of developing cancer, such as smoking, unhealthy diet, and a sedentary lifestyle [3].

Therapy for cancer can be in the form of radiation, surgery, hormone therapy, chemotherapy and targeted therapy. This cancer therapy aims to destroy cancer or limit disease progression and eliminate its symptoms. One of the primary therapies for both early-stage and advanced cancer is doxorubicin [4]. However, there are weaknesses in chemotherapy, the first line of damaged organs may also include those generated by continually dividing cells, such as skin, hair, and intestinal linings. The most frequent and obvious adverse effects, which can cause vomiting, nausea, diarrhea, and constipation, come from the gut. Prolonged therapy can result in extreme toxicity, seriously harming or failing several organs, and Multi-Drug Resistance (MDR) happens [5, 6]. So, there is a demand to find active and selective compounds to prevent and treat cancer.

Herbal treatment has been proposed and researched as an alternative to cancer treatment. One of the reasons it contains compounds that have cytotoxic effects. Natural anticancer compounds typically come from an alkaloid (such as vincristine and vinblastine) [7], flavonoids group [8], and steroid group [9]. One of the plants is known to have a cytotoxic effect, namely kratom leaf (*Mitragyna speciosa*).

Kratom's phytochemical characteristics have been thoroughly documented for many years. There are 79 secondary metabolites in kratom, along with a significant amount of alkaloids, flavonoids, terpenoids, triterpenoids, saponins, polyphenols, and secoirods [10]. Based on the research of Saidin, (2008) showed that the alkaloid content of mitragynine and kratom leaf extract has cytotoxic activity on SH-SY5Y nerve cells and MCL-5 lymphoblastoid cells. It has a high cytotoxic and antiproliferative effect against erythroleukemia and colon cancer [11]. Phytochemicals and their derivatives may increase therapeutic efficacy in cancer patients while reducing side effects. The combination of doxorubicin and an alkaloid extract from kratom leaf increased the drug's sensitivity in A549 lung cancer cells by 2.6 to 3.4 times, indicating that the two substances may cooperate to reduce the amount of doxorubicin administered during treatment [12]. Often, phytochemical substances work by regulating molecular signaling pathways connected to the onset of cancer. The immune system's regulation, apoptotic and proliferation of cells arrest, carcinogen suppression, proliferation inhibition, and inhibitory enzyme topoisomerase are examples of cytotoxic mechanisms [13, 14].

Researchers studying cancer have been intrigued by the activity of topoisomerase II, a target of effective anti-cancer medications like doxorubicin and etoposide [15]. These studies revealed that the majority of clinically active topoisomerase IIα inhibitors cause enzyme-mediated DNA damage [16]. Many rapidly reproducing cancer cells overexpress topoisomerase Iiα [17]. Therefore, it is thought to be a substantial therapeutic approach to produce topoisomerase IIα-targeting anticancer medicines by inhibiting topoisomerase IIα by triggering double-strand DNA breaks or preventing ATP degradation [18].

Previous studies have evaluated the cytotoxic activity of the kratom plant alkaloid on nerve, leukemia, colon, nasopharyngeal, and lung cancer cells *in vitro* [11, 12, 19]. However, there are no particular findings on the presence of active chemicals from kratom leaf with cancer-fighting mechanisms at the topoisomerase IIα receptor. Molecular docking was an effective method for finding putative bioactive substances in nature and finding out how mecanism these substances bind and interact with protein targets [20]. A combination of in silico techniques and chromatographic/spectroscopic studies can be used to identify the

bioactive chemicals found in the extract [21]. Liquid chromatography-mass spectrophotometry/mass spectrophotometry (LC-MS/MS) was used to identify the isolated chemicals [22]. This aims to determine the metabolite content of kratom leaves using LC-MS/MS and their activity to inhibit topoisomerase IIα were predicted using molecular docking. In silico predictions are required to identify chemical components from kratom leaves that are active against cancer and will eventually become medication candidates for cancer drugs or cancer chemopreventives.

MATERIALS AND METHODS

Plant material and sample preparation

Kratom leaf (*Mitragyna speciosa*) dried plant was obtained from suppliers in the Pontianak area, Indonesia. *Mitragyna speciosa* (Korth.) Havil has been identified as the dried plant, as confirmed by Materia Medica Batu. Kratom leaf dried plant powder was extracted by maceration method for 24 h using 2.5 l of 96% ethanol with 2 times repetitions. Additionally, the fractionation process was conducted following the methodology outlined by Sharma *et al.* (2019). The kratom leaf dry extract was reconstituted using 96% ethanol and then acidified with 10% HCl to achieve a pH of 2-3. The process of liquid-liquid extraction was performed using hexane in a 1:1 ratio. The hexane portion was isolated from the water portion, followed by separation of the water portion through alkalinization using a 10% Na_2CO_3 solution until a pH of 8-9 was achieved. The water portion is extracted from liquid-liquid with ethyl acetate in a 1:1 ratio. The ethyl acetate portion was isolated from the water portion and collected [23].

LC-MS/MS analysis

The LCMS/MS instrument was used to identify the chemicals present in the kratom leaves. The chromatography system uses an Ultra Performance Liquid Chromatography (UPLC) system. The specimen will be inserted into the liquid chromatography system using a 5 µl microsyringe and thereafter entered into the UPLC system. The column uses C18 (2.1 x 100 mm; 1.8 µm). ESI charge, a mass range of 50–1200 m/z with positive (+) mode. Furthermore, effect energy ranging from 4 to 60 electron volts, cone gas flow 0 l/h, and desolvation gas flow 793 l/h. Moreover, the ions generated by the detector were divided by the Q-ToF analyzer. A mobile phase consisting of Water+5 mmol Amonium Formic (phase A) and Acetonitril+0,05 % Formic acid (phase B) was employed with gradient method (0 min, 95:5 (A: B); 2 min, 75:25 (A: B); 3 min, 75:25 (A: B); 14 min, 0:100 (A: B); 15 min, 0:100 (A: B); 19 min, 95:5 (A: B); 23 min, 95:5 (A: B), and the flow rate was 0.2 ml/minute. For data collection and processing, the Masslynk V4.1 application was used to evaluate the chromatogram peaks. A literature and online database search was conducted to identify the m/z number for a specific molecular ion. Selection criteria for identified compounds are based on how well the compounds tested by LC-MS/MS match those on the library website or in other research articles in terms of their chemical formula, m/z, and fragmentation. The online database utilized by the library is https://hmdb.ca/.

Molecular docking tools

The hardware used is the ASUS X409FJ computer with Intel® Core™ i5 8265U @ 1.80 GHz Processor specifications and 4 GB Random Access Memory (RAM). The proteins were downloaded from the following website: https://www.rcsb.org/. Compound structures can be downloaded from the following website: https://pubchem.ncbi.nlm.nih.gov. The software used is ChemDraw 20.0 and Chem3D 20.0 to create the structure of the test ligand compounds, AutoDockTools 4.2.6 for the preparation of target macromolecules, AutodockVina 1.2.5 (https://pubs.acs.org/doi/10.1021/acs.jcim.1c00203) in the PyRx 0.8 system and Discovery Studio Visualizer v.19.1.0.18287 for in silico testing.

Preparation of protein

Molecular docking analysis was performed to explore the binding mode of compounds in the active site of the native ligand (Etoposide) from the structure of human (Homo sapiens) topoisomerase IIα enzyme (PDB: 5GWK) (https://www.rcsb.org/structure/5GWK). The protein structure of chain A from PDB 5GWK was constructed using AutoDock Tools. All uncommon residues and atoms of water were removed from the basic structure. Subsequently, the system underwent an upgrade wherein all absent hydrogen atoms and kollman charges were added. The protein receptor that had been created was thereafter exported in pdbqt format and directly placed into the workspace folders of PyRx.

Preparation of ligands

The structure of the compound as a result of LCMS analysis was obtained from the PubChem website in the form of SMILES. Created in a 3D model by copying SMILES into the Chem3D program. Optimized with MMFF94 and stored in. mol2 format and made into a test ligand using PyRx 0.8 in. pdbqt format.

Docking validation

The AutoDockVina software is used to perform molecular docking. The redocking procedure commences with the validation step, wherein the cocrystal ligand recovered from the receptor is employed as an experimental ligand, and its position is utilized as the binding site [24]. The Root mean Square Deviation (RMSD) number provides an indication of the validation outcomes [25]. Align the grid box at the center using a grid box size of 25 and binding site coordinates of x = 31.3837; y =-22.6558; z =-58,1512. Validation was considered valid if the RMSD was redocked and the crystallographic ligand superimposed was less than 2Å. Protein-ligand interaction visualization analysis was carried out using BIOVIA's Discovery Studio Visualizer.

RESULTS AND DISCUSSION

LC-MS/MS analysis

The metabolomic investigation showed an existence of polyphenols, terpenoid, steroids, alkaloids, fatty acid and flavonoids. This result showed in table 1 and the Base Peak Intensity (BPI) chromatogram of kratom leaf extract and sub-extracts partitioned using n-hexane and ethyl acetate as solvents can be seen in fig. 1. Based on the data presented in fig. 1, the percentage area of each compound's peak at a specific retention time can be observed. Fig. 1 shows that the peak with the highest area percentage in the ethanol extract is at a retention time (RT) of 7.43, which is predicted to be the mitragynine compound. This is in line with previous research conducted by Casey *et al.* (2015) [26] and Avula *et al.* (2015) [27] and Veeramohan *et al.* (2018) [28], where bioactive compound is mitragynine. The mitragynine molecule has a variable retention time among the three references, including the findings of this investigation; nevertheless, the m/z value across all references and the research findings is the same, at 399.2 m/z. The compound that dominates in kratom leaves is mitragynine. Because of its varied biochemical and pharmacological effects, it has potential applications in various therapeutic domains, including analgesic, antitussive, adrenergic, antimalarial, and cancer treatment [29].

The peaks with the highest area percentage in the chromatogram of the hexane and ethyl acetate sub-extracts of kratom leaves, respectively, were at retention times (RT) 14.70 and 7.03, which were predicted to be the compounds linolenic acid and mitragynine. Lin *et al.* (2019), who identified bioactive compounds linolenic acid with retention time 21,54 min with 278 m/z [30]. Linolenic acid acid molecules have different retention times between the references and the findings of this study but have relatively the same m/z values. Linolenic acid *in vitro* and in vivo has been reported to have proliferation and apoptosis activity. Mitragynine also widely reported have activity in nerve cancer, leukemia and colon cancer [18, 11, 31]. All of the detected compounds, including linolenic acid and mitragynine, have been inspected in the https://hmdb.ca/database. They all exhibit fragmentation patterns, m/z, and chemical formulae that match. The results in table 1, the alkaloid group compounds were the dominant compounds found in kratom leaves. In line with Veeramohan *et al.* (2018) findings [28], the majority of the molecules found in the metabolomics data of *Mitragyna speciosa* leaves using LC-ESI-TOF-MS are alkaloid compounds.

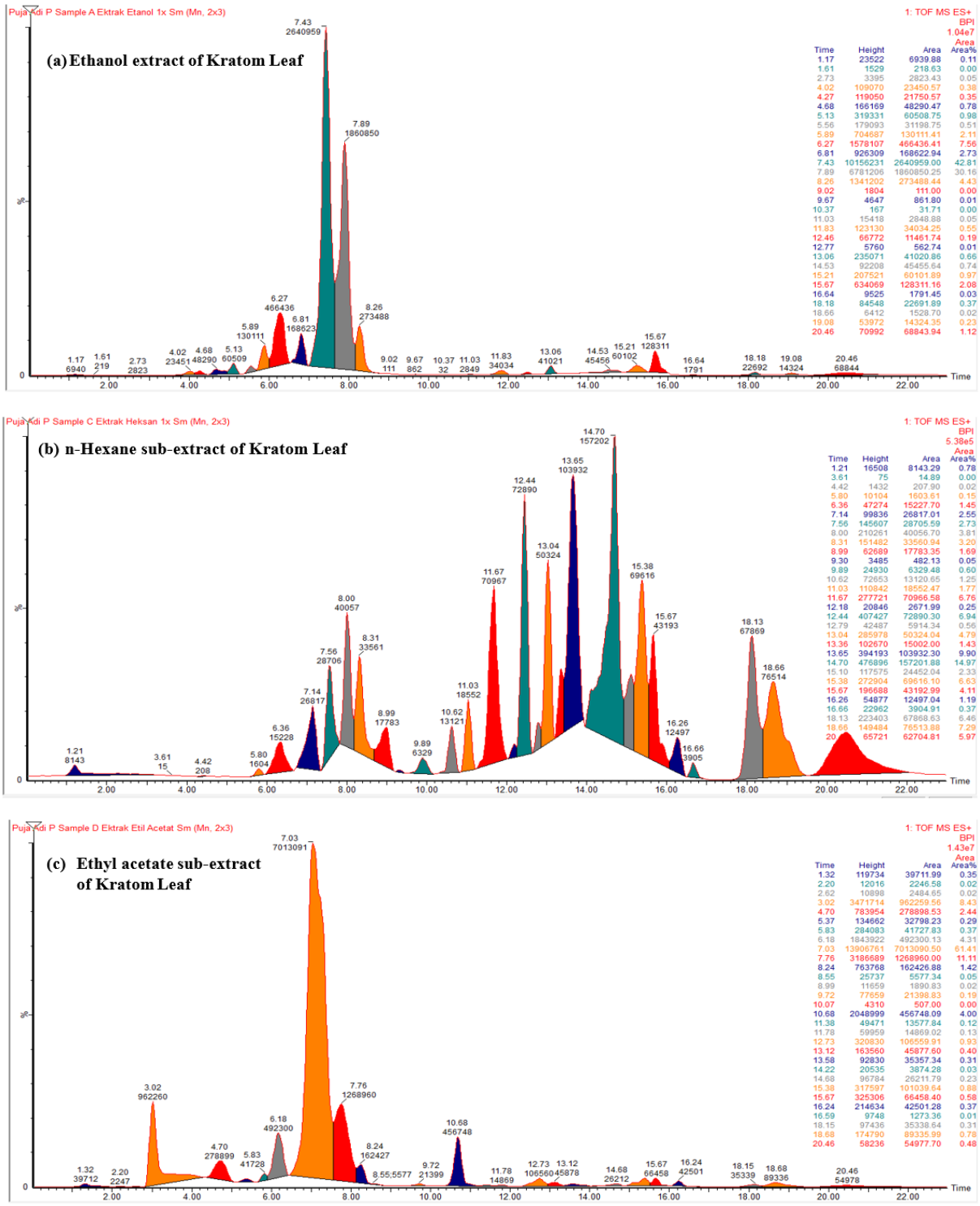

Fig. 1: Basic peak intensity chromatogram using LC-MS/MS of ethanol (a), hexane (b) and ethyl acetate (c) extracts of kratom (*M. speciosa*) leaves

Molecular docking

The molecular docking validation results were declared valid with an RMSD value of 1.0835Å. An image of the overlay results between the original ligand and the crystallographic ligand can be seen in fig. 2. Molecular docking assays were carried out using the same size and position of grid boxes. The parameters observed to determine the affinity of the ligand for the receptor are binding free energy (ΔG) value, amino acid residues, and the amount of hydrogen bonds [32]. The results of a molecular docking study of topoisomerase IIα targets with Autodock Vina can be observed in table 2. The more negative the ΔG score indicates a powerful complex is formed between the ligand and the standard. From these conformations, the top ranking is chosen, which has the lowest ΔG bind value, and then looks at the interactions, amino acid residues, and the amount of hydrogen bonds.

The native ligand (Etoposide) has several key contacts between the ligand and vicinal residues that provide a stable and constrained conformational system, thus supporting the compound's action as a

topoisomerase II inhibitor. The following amino acid residues are present in the interaction of the 5GWK protein with the native ligand: DT6, DG13, Met766, Asp463, Gly462, and Arg487. In the crystal structure of the ternary topoisomerase II/DNA/etoposide complex, the drug molecule is stabilized by interactions with Asp463 and Arg487 [33]. The Asp 463 and Arg487 residues are located in the active pocket and play an important role because they contribute to the stability of the topoisomerase II complex, thereby increasing the affinity of the compound. In addition, adjacent DNA bases also contribute to the stabilization of the complex. Based on research conducted by Arencibia *et al.* (2020), the results of molecular dynamics simulations show the role of Arg487 in stabilizing the drug at the cleaved site [34]. Reviewed from monitoring the distance between carbon atoms of the native ligand, it was found that the distance remained below 6 Å for 99% of the simulations.

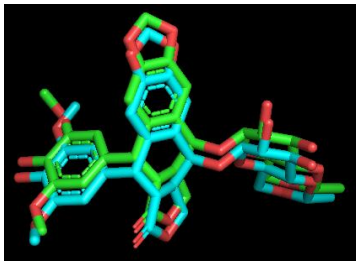

Fig. 2: Native ligand overlay of macromolecular topoisomerase IIα docking method validation with PyMOL-green (crystallography results) and blue (re-docking results)

Table 1: Data concerning compounds identified on ethanol, hexane, and ethyl acetatea extract of kratom (*M. speciosa*) leaves

Tentative compound	Elemental composition	Calculated m/z	Experiment m/z	Major Ions in MS/MS Spectra	Ethanol RT, min	Hexane RT, min	Ethyl acetate RT, min	Ref.
Chlorogenic acid	$C_{16}H_{18}O_9$	355.1029	355.1023	303; 287; 163; 135	3.94	-	-	[30]
Procyanidin	$C_{30}H_{26}O_{12}$	579.1503	579.1504	561; 427; 409; 289	4.04	-	-	hmdb
Epicatechin	$C_{15}H_{14}O_6$	291.0869	291.0855	273; 147; 139; 123	4.27	-	-	hmdb
Tripropylamine	$C_9H_{22}N$	144.1752	144.1754	142; 110; 102; 89	-	4.42	-	hmdb
O-Coumaric acid	$C_9H_8O_3$	165.0552	165.0556	151; 123; 118; 95	4.60	-	-	[30]
Rutin	$C_{27}H_{30}O_{16}$	611.1612	611.1629	465; 387; 369; 303	4.68	-	-	[30]
Rotundifoline	$C_{22}H_{28}N_2O_5$	401.2076	401.2086	385; 369; 341; 153	6.11	-	4.73	hmdb
11-Hydroxyyohimbine	$C_{21}H_{26}N_2O_4$	371.1971	371.1992	301; 287; 257; 165	4.88	-	4.88	hmdb
5(S)-5-carboxystrictosidine	$C_{28}H_{34}N_2O_{11}$	575.2241	575.2289	502; 371; 287; 182	5.10	4.97	-	hmdb
Tofisopam	$C_{22}H_{26}N_2O_4$	383.1971	383.1929	351, 267; 182; 160	5.89	-	-	hmdb
Isorhynchophylline	$C_{22}H_{28}N_2O_4$	385.2127	385.2120	353; 241; 160; 130	6.36	6.36	3.02	[29]
7-hydroxymitragynine	$C_{23}H_{30}N_2O_5$	415.2233	415.2237	401; 321; 271; 190;	7.12	7.14	-	[27]
Corynantheidine	$C_{22}H_{28}N_2O_3$	369.2178	369.2180	337; 328; 204; 144	7.19	-	-	[28]
Mitragynine	$C_{23}H_{30}N_2O_4$	399.2284	399.2296	367; 351; 238; 174	7.41	7.98	7.10	[26-28]
Dihydroactinidiolide	$C_{11}H_{16}O_2$	181.1229	181.1240	163; 123; 118; 82	-	9.02	-	hmdb
Myrsinone	$C_{17}H_{26}O_4$	295.1909	295.1912	277; 179; 137; 118	-	-	9.76	hmdb
Hexosylsphingosine	$C_{24}H_{47}NO_8$	478.3380	478.3380	343; 240; 181; 123	-	9.89	-	hmdb
Polyneuridine aldehyde	$C_{21}H_{22}N_2O_3$	351.1709	351.1709	319; 291; 261; 179	-	-	10.58	hmdb
Dehydrophytospingosine	$C_{18}H_{37}NO_3$	316.2852	316.2837	298; 280; 191; 142		10.62		hmdb
Phytosphingosine	$C_{18}H_{39}NO_3$	318.3008	318.3016	300; 279; 183; 142	11.03	11.03	-	hmdb
3-oxo-12,18-ursadien-28-oic acid	$C_{30}H_{44}O_3$	453.3369	453.3366	399; 318; 259; 207	11.12	-	-	hmdb
Bolandiol	$C_{18}H_{28}O_2$	277.2168	277.2170	259; 142; 123; 105	11.89	13.65	11.78	hmdb
Androsterone	$C_{19}H_{30}O_2$	291.2234	291.2353	282; 277; 259; 142		15.38	13.58	hmdb
P,P-Dioctyldiphenylamine	$C_{28}H_{43}N$	394.3474	394.3459	325; 279; 256; 142	15.21			hmdb
Linolenic acid	$C_{18}H_{30}O_2$	279.2324	279.2318	261; 243; 181; 118		14.70		[30]

Table 2: Results of ΔG values and ligand-protein interactions

Compunds	Δ Gbinding (kkal/mol)	Residues involved in interaction of ligand-protein						Similarity of amino acid residues	
		DT9	DG13	Met766	Asp463	Gly462	Arg487	Etoposide	Doxorubicin
Native ligand (Etoposide)	-12.825±0.05	✓	✓	✓	✓	✓	✓	-	-
Doxorubicin	-11.58±0.49		✓	✓	✓	✓	✓	66.67 %	-
(5S)-5-Carboxystrictosidine	-10.74±0.05	✓			✓			33.34 %	25.00%
3-Oxo-12-18-ursadien-28-oicacid	-9.34±0.05							0.00 %	0.00%
7-Hydroxymitragynine	-8.38±0.04			✓				16.67 %	0.00%
11-Hydroxyyohimbine	-8.65±0.10	✓						16.67 %	0.00%
Androsterone	-8.42±0.08							0.00 %	0.00%
Bolandiol	-8.2±0.00	✓					✓	33.34 %	25.00%
Chlorogenic Acid	-8.56±0.13		✓		✓		✓	50.00 %	75.00%
Corynantheidine	-8.94±0.13	✓	✓				✓	50.00 %	50.00%
Dehydrophytospingosine	-5.58±0.23	✓					✓	33.34 %	25.00%
Dihydroactinidiolide	-6±0.00							0.00 %	0.00%
Epicatechin	-9.1±0.00	✓	✓		✓		✓	66.67 %	75.00%
Hexosylsphingosine	-7.08±0.22	✓	✓			✓	✓	66.67 %	75.00%
Isorhynchophylline	-8.74±0.61	✓	✓				✓	50.00 %	50.00%
Linolenic acid	-5.74±0.21	✓	✓				✓	50.00 %	50.00%
Mitragynine	-8.98±0.13	✓	✓				✓	50.00 %	50.00%
Myrsinone	-6.82±1.16	✓	✓					16.67 %	25.00%
o-Coumaricacid	-5.9±0.00		✓				✓	50.00 %	50.00%
p,p-Dioctyldiphenylamine	-6.9±0.24		✓				✓	50.00 %	50.00%
Pholcodine	-8.825±0.05	✓		✓			✓	50.00 %	25.00%
Phytosphingosine	-5.46±0.15				✓		✓	16.67 %	50.00%
Polyneuridinealdehyde	-8.02±0.44		✓	✓				16.67 %	25.00%
Procyanidin	-10.1±0.00	✓	✓	✓				50.00 %	25.00%
Rotundifoline	-9.32±0.29							0.00 %	0.00%
Rutin	-10.78±0.27	✓	✓		✓		✓	66.67 %	75.00%
Tofisopam	-8.98±0.04	✓	✓					16.67 %	25.00%

Table 2 shows a comparison of the amino acid residues of doxorubicin and natural ligands (etoposide) to metabolite chemicals in kratom leaves. When comparing the amino acid residue interactions of all evaluated ligands to those of native ligands or controls, comparative similarity of amino acids demonstrates how similar they appear. Doxorubicin, hexosylsphingosine, isorhynchophylline, and rutin had the highest percentage of amino acid residue similarity (66.67%) when compared to the native ligand etoposide, according to the results in table 2. When compared to the doxorubicin control, the test chemicals rutin, epicatechin, hexosylsphingosine, and chlorogenic acid have the highest percentage of amino acid residue similarity, 75.00%. In a number of these compounds, the predominant amino acid residues are DG13 and Arg487. The likelihood that the test ligand will exhibit the same activity as the native cocrystal ligand increases with the similarity of amino acid residues [35].

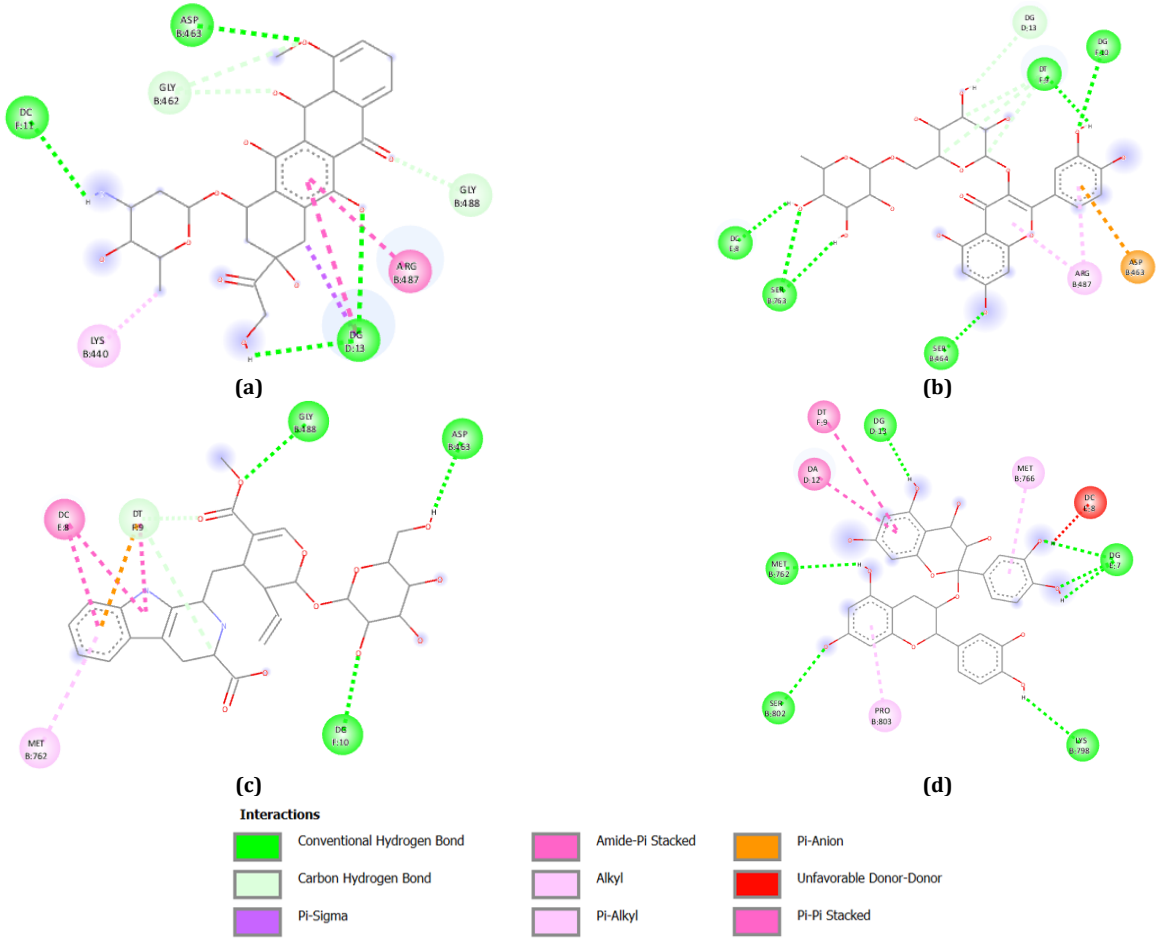

Fig. 3: Interactions of compounds (a) doxorubicin; (b) routine; (c)(5*S*)-5-Carboxystrictosidine; and (d) Procyanidin against Topoisomerase Iiα

As a positive control, Doxorubicin has a ΔG value of-11.58 kcal/mol. Hydrogen bonds formed in Doxorubicin with topoisomerase IIα occur at residues DG13, Asp463, Gly488, and Gly462 and interact with residues Arg487 and Lys440. The dominant metabolites identified from the ethanol extract, the hexane and ethyl acetate sub-extracts of kratom leaves were linolenic acid and mitragynine. On the other hand, this substance does not have a great potential for topoisomerase IIA receptor inhibition, with mitragynine and linolenic acid having ΔG values of-8.98 and-5.74, respectively. As per the molecular docking approach, each chemical's activity capabilities are virtually screened. This enables a substance to construct a ligand-receptor complex based on binding energy without considering its content. The "lock-key" paradigm describes the precise location of a compound's binding to a macromolecule's stiff active site [36]. Despite its lower molecular docking activity to topoisomerase IIa receptors, linolenic acid has been shown to exert apoptotic and proliferative effects on cancer cells. There have also been numerous reports of mitragynine's effects on colon cancer, leukemia, and nerve cancer [18, 11, 31]. Three compounds with the lowest ΔG values were selected, namely Rutin, (5S)-5-Carboxystrictosidin and Procyanidin, which were then observed for their interactions with amino acid residues. The rutin compound has three hydrogen bonds and a ΔG value of-10.78 kcal/mol. Rutin interacts with four residues that are the same as the original ligand of the topoisomerase IIα protein, namely DT9, DG13, Asp463, and Arg487. These results indicate that Rutin interacts with Asp463 and Arg487, which are amino acid residues that play an important role in inhibiting topoisomerase IIα. *In vitro*, rutin has been reported to have the ability to inhibit the proliferation of seven breast cancer cell lines. Rutin was identified as a type II topoisomerase inhibitor in a study conducted by Heba *et al.* (2017) [37].

Furthermore, (5S)-5-Carboxystrictosidine with a ΔG value of-10.74 kcal/mol interacts to form two hydrogen bonds to residues DT9 and Asp463. (5S)-5-Carboxystrictosidine belongs to the class of monoterpenoid indole alkaloids whose anticancer activity has never been reported. According to the chemical class, it has been observed by in silico that indole alkaloids found in kratom leaves have the ability to block estrogen receptor alpha and induce apoptosis. This is achieved by disrupting the connection between p53 and MDM2, then reactivating p53 activity [38]. Qin *et al.* (2022) found indole alkaloid chemicals can inhibit Bcl-2 that trigger apoptosis in MCF-7 cell [39].

Finally, procyanidin forms a hydrogen bond with residue DG13 and interacts with residue DT9 and Met766 having a ΔG value of-10.1 kcal/mol. Procyanidin resulted in inhibition MCF-7 and MDA-MB-468 cancer cell[40]. Previous research indicates that p21 expression may be upregulated in order to mediate the cell cycle arrest caused by procyanidin [41, 42]. Procyanidin-induced G0/G1 cell cycle arrest in human esophageal cancer OE-33 cells was not significantly affected by p21 expression knock-down using p21-specific si-RNA [43]. Procyanidin was discovered to be one of the most effective inhibitors of KDNA decatenation mediated by DNA-topoisomerase-II (Topo-II) [44]. Procyanidin's potential as a chemopreventive agent is indicated by its ability to modify gene expression in animal models. For the purpose of developing new cancer treatments, it will be beneficial to thoroughly identify the mechanism targets of rutin, (5S)-5-carboystrictosidine, and procyanidin action. The similar binding of the same residue with both Doxorubicin and the natural ligand results in the prediction that the three compounds mentioned above will exhibit the same action as Doxorubicin, serving as positive controls. Limitations of molecular docking studies include focusing solely on a compound's activity when interacting with proteins and neglecting absorption, distribution, metabolism, and excretion. Consequently, an ADMET study is required for additional research, which can be conducted, for example, by using the SWISS ADME website [45], which influences the drug's stability or activity. A thorough discovery of chemopreventive targets and biomarkers for the three substances mentioned above will greatly aid in the future advancement of cancer treatment.

CONCLUSION

The LC-MS/MS study of kratom leaf extract revealed the presence of 25 different components, which include polyphenols, terpenoids, steroids, alkaloids, fatty acids, and flavonoids. The peaks with the highest area percentage in the chromatogram of the ethanol extract, the hexane and ethyl acetate sub-extracts of kratom leaves were linolenic acid and mitragynine. On the other hand, this substance does not have a great potential for topoisomerase IIA receptor inhibition. The top three compounds that showed the strongest ability to inhibit topoisomerase IIα were rutin, 5(S)-5-carboxystrictosidine, and procyanidin. These compounds had energy binding scores (ΔG) of 10.78 kcal/mol, 10.74 kcal/mol, and 10.10 kcal/mol. The findings indicated the alkaloid group compounds were the dominant compounds found in kratom leaves and shown a robust capability to inhibit topoisomerase IIα. Nevertheless, since molecular docking only accounts for a compound's activity when it interacts with proteins and ignores aspects of chemical, physical, and other biological aspects that affect the stability or activity of the drug, these results still require validation through *in vitro* or in vivo research. Further research on the isolation of active chemical compounds from screening results with molecular docking on Topoisomerase IIa against cancer cells is needed to explain their potential pharmacological activity and mechanism of action in cancer treatment.

FUNDING

The authors express their gratitude for the support received from the Pendidikan Magister Menuju Doktor untuk Sarjana Unggul (PMDSU) scholarship provided by the Ministry of Research, Technology, and Higher Education Indonesia. The research and higher education activities were financially supported by the Ministry of Research and Higher Education of Indonesia [PMDSU 2022 (NKI: 085/E5/PG.02.00. PT/2022) (NKT: 898/UN3.15/PT/2022)].

AUTHORS CONTRIBUTIONS

All the authors have contributed equally and approved the final version of the manuscript. Sukardiman and Widyowati were in charge of the study's conceptualization and design, offering the fundamental structure and goals. Priatna and Rahmah were important in gathering and organizing the data required for analysis through their meticulous data acquisition. The development of the manuscript involved all authors working together to construct the language, integrate findings, and refine the content to guarantee scientific rigor and clarity.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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