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Original Research Article

In Silico Evaluation of the Physicochemical, Pharmacokinetics, and Toxicity Profiles of Sesquiterpene Lactones of South African Leaf (*Vernonia amygdalina* Delile)

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ABSTRACT

Cancer is a disease caused by malignant cell growth. Chemotherapeutic agents are still the primary option for cancer treatment. Various efforts to develop new treatment methods are needed for more effective cancer therapy. Cyclophosphamide is one of the essential agents used for treating cancers. The sesquiterpene lactone compounds in *Vernonia amygdalina* Delile, (South African leaf) contribute to its anticancer pharmacological effects in different cancers. This study aims to determine the pharmacokinetics and toxicity profiles of various sesquiterpene lactone compounds in the South African leaf. This research was initiated by a search for the physicochemical properties and Canonical Simplified Molecular Input Line Entry System (SMILES) code of each compound with the assistance of PubChem, followed by computational processing with the aid of the pkCSM and ProTox-II tools. This study involves a comparative analysis of the compounds' physicochemical properties, pharmacokinetics, toxicity profile and cyclophosphamide as the standard anticancer drug. The results showed that the physicochemical properties of the sesquiterpene lactone compounds in *Vernonia amygdalina* leaf had met Lipinski's rule of five conditions. The pharmacokinetics and toxicity profiles were similar or better than cyclophosphamide. Hydroxyvernolide has the best physicochemical, pharmacokinetics, and toxicity profile of the sesquiterpene lactones of the South African *Vernonia amygdalina* and cyclophosphamide.

***Keywords*:** *Vernonia amygdalina* Delile, Sesquiterpene lactone, *In silico*, Physicochemical, Pharmacokinetics, Toxicity.

Introduction

Pharmacokinetics is the study of the entire process experienced by drug molecules from the time it enters the body until the drug is excreted from the body. The drug action processes in pharmacokinetics are sequentially absorption, distribution, metabolism, and excretion, which affects the half-life ($T_{½}$), peak time (T_{max}), the onset of action, and duration of action.[1] Toxicity is the ability of a material to have a toxic or poisonous effect for a certain period due to chemical, physiological interactions in the body. Toxicity testing of material is specific or alternative to determine risk factors.[2]

The process of introducing new drugs to the market is very complex, including preclinical trials and clinical trials to prove safety, efficacy, and quality.[3]The safety of a developed drug compound must be verified by its low toxicity during preclinical and clinical trials.[4] Scientific and economic pressures to minimize chemical and biological experiments and quickly and precisely screen new compounds with better pharmacokinetic and toxicity profiles have led to accelerated processes and developments in computational chemical engineering in the pharmaceutical industry.[5]

The South Africa Leaf (*Vernonia amygdalina* Delile) is a plant from the Asteraceae family that has its origin in Africa, including Zimbabwe and Nigeria. It grows best in tropical climates and can be grown wild or planted. *Vernonia amygdalina* leaf has various pharmacological effects due to its numerous phytochemical constituents, including sesquiterpenes, triterpenes, flavonoids, alkaloids, saponins, tannins, and glycosides.[6] The content of sesquiterpene lactone compounds contained in the leaf of the South African Leaf, *Vernonia amygdalina*, has been shown to contribute to its anticancer activity against various cancers, namely lung cancer, colorectal cancer, cervical cancer, and breast cancer.[7,8] The sesquiterpene lactone compounds present in *Vernonia amygdalina* Leaf are vernodalol, vernodalin, vernolepin, vernomygdin, vernolide, hydroxyvernolide.[9]

Cancer is excessive cell proliferation, generally embryonic, which invades and destroys the surrounding tissue. Cancer treatment with chemotherapeutic agents is still the primary option for most cancer patients.[10] However, the existence of multiple drug resistance mechanisms results in reduced efficacy of chemotherapy drugs.[11]Various efforts to develop new treatment methods are needed for more effective cancer therapy. One of them is cyclophosphamide.[12] Alternative medicines, such as medicinal plants used to treat degenerative diseases, can reduce side effects.[13]

An excellent pharmaceutical compound is not enough to be efficacious but must also have a good pharmacokinetics and safety profile. In silico Physicochemical, pharmacokinetic, and toxicity analysis of the sesquiterpene lactone compounds of the South Africa Leaf have not been reported previously. This study aims to determine

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the pharmacokinetics and toxicity profile of various sesquiterpene lactone compounds present in the South Africa Leaf.

Materials and Methods

Hardware

The hardware used in this study is a laptop with a Processor specification with the type Advanced Micro Devices, Inc. (AMD) Ryzen 7 - 3700U with Radeon Vega Mobile Gfx 2.30 GHz, Random Access Memory (RAM) with a capacity of 8 GB, and Read-Only Memory (ROM) capacity of 512 GB.

Software

The software used in this study is PubChem (https://pubchem.ncbi.nlm.nih.gov) for molecular structure of analysis of compounds, pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=compound_input) for pharmacokinetic analysis and toxicity analysis.

Methods

Prediction of the physicochemical, pharmacokinetic, and toxicity properties of the South Africa sesquiterpene lactone compounds (vernodalol, vernodalin, vernolepin, vernomygdin, vernolide, hydroxyvernolide) as test compounds and cyclophosphamide as standard oral chemotherapy begins with a search for the physicochemical properties and Canonical Simplified Molecular Input Line Entry System (SMILES) code with the assistance of PubChem website, followed by computational processing using pkCSM and ProTox-II websites. The process ends with an analysis of the computational data generated on the compounds' physicochemical, pharmacokinetic, and toxicity properties compared to cyclophosphamide as the standard anticancer drug.

Results and Discussion

The structure of the compound was obtained from PubChem in the Simplified Molecular Input Line Entry System (SMILES) format for physicochemical, pharmacokinetic, and toxicity analysis using pkCSM and ProTox-II (Figure 1).

Preliminary analysis of the physicochemical properties was carried out to assess the fulfillment of Lipinski's rule of five of the compounds to be analyzed. The physicochemical properties analyzed include hydrogen bond donors, hydrogen bond acceptors, molecular mass, and partition coefficient obtained from PubChem, as shown in Table 1.

Lipinski's rule of five requires that a drug lead have a molecular mass of less than 500 Daltons, a partition coefficient of not more than five, five hydrogen bond donors, and not more than 10 hydrogen bond acceptors. The above analysis is known as Lipinski's rule of five because all values are multiples of the number five. Active drugs with oral administration must comply with Lipinski's rule of five with no more than one violation.[14] The results showed that both South Africa Leaf sesquiterpene lactone compounds and cyclophosphamide met Lipinski's rule. Both drug candidates possess good oral administration and intestinal absorption. The number of rotating bonds is a parameter of the flexibility of a compound, widely used in the process of discovering new drugs or drug candidates. A compound is declared to have good permeability and bioavailability if it has no fewer than 15 rotating bonds.[15] The results showed that both South Africa Leaf sesquiterpene lactone compounds and cyclophosphamide had a range of 1–8 rotating bonds, meaning that all of them have good flexibility, permeability, and bioavailability.

Pharmacokinetic and toxicity analysis was carried out by pkCSM using several parameters: for absorption (intestinal absorption and skin permeability), for distribution (distribution volume, fraction unbound, blood-brain barrier permeability, and central nervous system permeability), for metabolism (CYP2D6 substrate CYP3A4 substrate CYP2D6 inhibitor, and CYP3A4 inhibitor), and excretion (total clearance and renal organic cation transporter substrate). Toxicity parameters investigated include Ames toxicity test, hERG I inhibitor, hERG II inhibitor, median lethal dose (LD50), lowest observed adverse effect level (LOAEL), and hepatotoxicity. Other parameters, including hepatotoxicity, carcinogenicity, and mutagenicity, were analyzed with ProTox-II (see Table 2).

Compounds with more than 70% absorption are declared as drugs with good absorption, while those with less than 30% are reported as poorly absorbed drugs. The intestine is the leading site for absorption of the main drug in oral drug administration.[16]The results showed that the predicted intestinal absorption values for both South Africa Leaf sesquiterpene lactone compounds and cyclophosphamide were in the range of 75% to 100%.

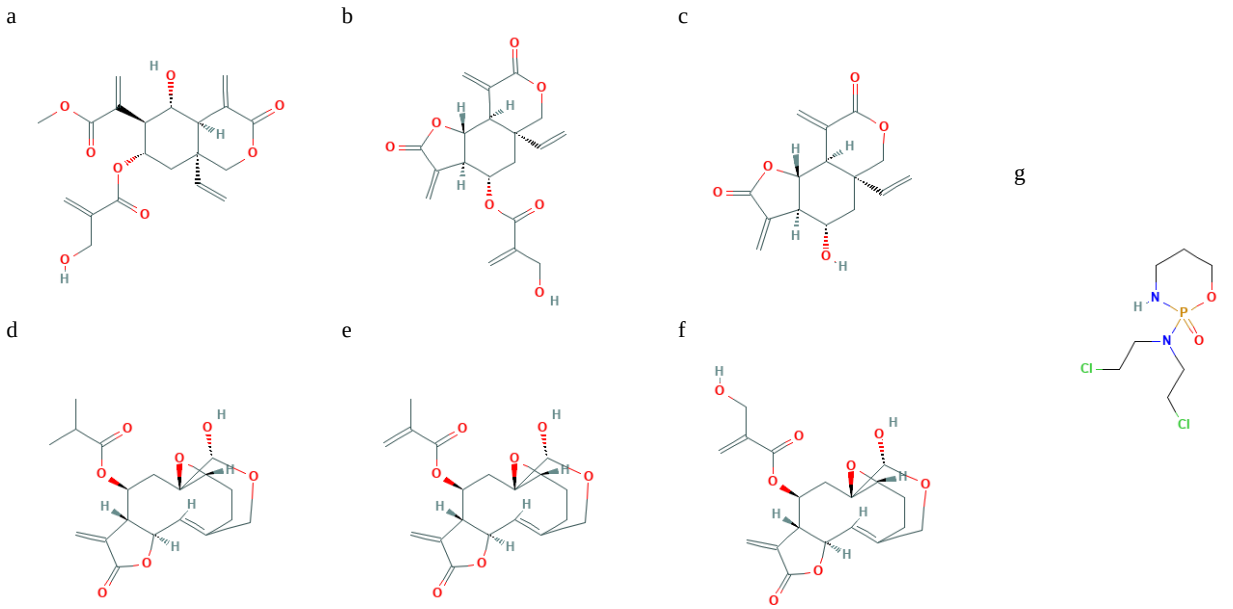

Figure 1: Structure of the South Africa Leaf Sesquiterpene Lactone Compounds and Cyclophosphamide used for Pharmacokinetic and Toxicity Analysis

*a: vernodalol; b: vernodalin; c: vernolepin; d: vernomygdin; e: vernolide; f: hydroxyvernolide; g: cyclophosphamide

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The predicted intestinal absorption values indicate that all of these compounds have good absorption.

A compound is declared to have good skin permeability with a value of less than -2.5. The results showed that the predicted skin permeability value (log Kp) for compounds was in the range of -3.867 to -2.908, while the predicted skin permeability value for cyclophosphamide was -2.281. This comparison shows that South Africa Leaf compounds have better skin permeability than cyclophosphamide.

The volume of distribution is the theoretical volume of the total dose of a drug required to be evenly distributed to give the same concentration in the blood plasma. A high volume of distribution indicates that the drug is distributed at high concentrations in tissues and low concentrations in plasma.[17]A compound is categorized as having a low volume of distribution if the logVD value is less than -0.15 and having a high distribution volume if the logVD value is less than 0.45.[18.] The results showed that the predicted logVD value of the compounds ranged from -0.236 to 0.198 (vernodalin being the least and hydroxyvernolide the highest), while the predicted logVD value for cyclophosphamide was -0.198. The predicted unbound fraction for vernodalin and vernodalol did not show a significant difference compared to cyclophosphamide, which means both have a similar distribution profile.

The concentration of unbound drug in systemic circulation determines the concentration of the active drug that could exert a pharmacological effect.[19] The results showed that the predicted unbound fraction for the test compounds was ranged from 0.404 to 0.551 (vernomygdin was the least while hydroxyvernolide the highest), while the predicted unbound fraction for cyclophosphamide was 0.586. The predicted unbound fraction for hydroxyvernolide was not significantly different from cyclophosphamide, suggesting a similar pharmacological effect with the standard anticancer drug cyclophosphamide.

The ability of a drug to pass through the blood-brain barrier is an essential parameter of the toxicity of a drug or its efficacy in the brain.[20] This quality or permeability is expressed as logBB value, which is the logarithmic ratio of the drug concentration in the brain to the drug concentration in plasma. The greater the logBB value, the greater the drug penetrability into the brain.[21]At a logBB value of 0.3, the compound can easily penetrate the blood-brain barrier and moderate penetration at logBB < -1.0.[22] The results showed that the logBB values for the compounds and cyclophosphamide were in the range of -0.684 and 0.195. The overall predicted logBB value is in the range of -1.0 and 0.3, suggesting that the test compounds have moderate BBB penetration.

In addition to the logBB value, there is another gold standard for measuring permeability in the brain, namely the logPS value. The logPS value is considered a more informative data point.[23]The drug permeability into the brain and the central nervous system is expressed as logPS value. It is the logarithmic ratio of permeability surface-area product to the central nervous system. The higher the logPS value, the higher the drug penetration ability.[24] The compound is considered to easily penetrate the central nervous system if the logPS value is greater than -2 and less likely to penetrate the blood-brain barrier if logPS is less than -3.[25] The results showed that the logPS values for both the test compounds and cyclophosphamide were in the range of -3.179 and -2.975. The overall predicted logPS value for the test compounds is less than -3 (except vernolepin), indicating that they possess moderate penetration into the central nervous system.

Metabolic enzymes in the liver that act as biomarkers to determine the effect of drug response are Cytochrome P450 (CYP450) enzymes, which oxidatively metabolize endogenous substrates and foreign compounds. There are 10 types of Cytochrome P450 (CYP450) enzymes in the human liver, CYP1, CYP2 and CYP3 isoenzyme families involved in drug metabolism. The most dominant types of Cytochrome P450 (CYP450) enzymes found in humans are CYP2D6 (20.0%) and CYP3A4 (30.2%).[26]

The Cytochrome P450 enzymes metabolize drugs (substrates) into different metabolites, which can easily be excreted.[27]The results predict that the test compounds are not CPY2D6 and CYP3A4 substrates, except vernodalin, vernomygdin, and vernolide. Thus, they are predicted not to be rapidly metabolized or quickly excreted. Inhibition of Cytochrome P450 (CYP450) enzymes can lead to clinically adverse drug interactions.[28] If a drug is not a substrate in the metabolic process by the cytochrome P450 enzyme, the process of drug washout from the body becomes slower, and the dose of the drug can be minimized.[29] The results predict that the test compounds do not inhibit CPY2D6 enzymes and CYP3A4 enzymes. Hence these compounds may not cause clinically adverse drug interactions.

The total clearance (TC) parameter is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion through the kidneys). TC is related to bioavailability, and it is essential to determine the dose level in achieving steady-state concentrations.[30] The results showed that the predicted total clearance value (log mL per minute per kg body weight) for the test compounds was in the range of 0.665 to 1.267, while the predicted total clearance value for cyclophosphamide was 0.628. The predicted TC value shows that the test compounds have faster excretion rates than cyclophosphamide.

The kidney has an organic cation transporter (OCT). This transporter plays a vital role in the disposition and excretion of drug compounds. The OCT plays a role in drug entry by bridging the transport of several cation compounds from basolateral to apical renal tubular cells.[31] The isoform organic cation transporter 2 (OCT2) is primarily responsible for drug accumulation in the kidney. The isoform 1 (OCT1) is majorly accountable for drug accumulation in the liver.[32] Drug compounds which are organic cation transporter substrates in tissues, can cause drug toxicity in certain organs. The prediction results indicate that the test compounds and cyclophosphamide are not substrates for organic cation transporters (OCT), so they do not cause organ toxicity.

A mutagenic test is a primary screening test to determine the possibility of mutagenicity of a drug compound. The Ames test is one of the methods used to detect the mutagenic effect of a drug substance based on a reverse mutation system.

Table 1: Results of physicochemical properties of South Africa Leaf (*Vernonia amygdalina* Delile) Sesquiterpene Lactone Compounds and Cyclophosphamide

Compounds	Molecular Mass	Partition Coefficient	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Rotatable Bonds
Vernodalol	392.4	1.2	2	8	8
Vernodalin	360.4	1.6	1	7	5
Vernolepin	276.3	1.3	1	5	1
Vernomygdin	364.4	1.0	1	7	3
Vernolide	362.4	0.9	1	7	3
Hydroxyvernolide	378.4	–0.3	2	8	4
Cyclophosphamide	261.1	0.6	1	4	5

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Based on several studies, mutagenic substances may be carcinogenic.[33] Mutations are permanent changes in genes that may be responsible for many genetic disorders that result in certain cancers or syndromes.[34] Cancer is a disease characterized by malignant cells/tissues that grow quickly and uncontrollably and spread to other places in the patient's body.[35] From this study, it is predicted that vernolide and hydroxyvernolide have negative Ames toxicity. Additionally, vernodalol, vernodalin, and vernolepin were negative for mutagenicity and carcinogenicity. However, vernomygdin and cyclophosphamide were positive in the measure of Ames toxicity, mutagenicity, and carcinogenicity.

Cardiotoxicity is used for heart damage and dysfunction resulting from exposure to toxic chemical compounds or drug compounds.[36]The cardiotoxicity of the drug arises due to inhibition of the human Ether-a-go-go related gene (hERG) that causes blockage of potassium ion channels. The blockage causes a prolongation of the QT interval of the heart, a severe life-threatening cardiac side effect.[37] Hepatotoxicity is injury or damage to the liver caused by chemicals or drugs. Hepatotoxicity can be caused by the use of an inappropriate type or dose of medicine.[38]The results of the prediction of cardiotoxicity showed that both the test compounds and cyclophosphamide did not inhibit human Ether-a-go-go related gene (hERG). The lowest observed adverse effect level (LOAEL) is the lowest concentration of a substance or drug that can cause adverse effects. The lower the value of LOAEL, the more toxic the compound.[39] The prediction results for the LOAEL showed that the dose range for the compounds was 1.107 mg per kg body weight to 2.087 mg per kg body weight, while cyclophosphamide had LOAEL of 0.518 mg per kg body weight. Overall, the LOAEL obtained for the test compounds were higher than cyclophosphamide, indicating that the compounds were less toxic than cyclophosphamide. All the South Africa Leaf sesquiterpene lactone compounds are predicted not to cause skin sensitization, while cyclophosphamide is predicted to cause skin sensitization. Thus, South Africa Leaf sesquiterpene lactone compounds may be safer for the skin.

The median lethal dose (LD50) of a substance or drug is the amount that causes mortality in 50% of test animals in an experiment. The lower the LD50 value, the lower the safety of the compound.[40] Prediction results for the LD50 indicate that the dose range for the compounds was 556.469 to 1,494.301 mg per kg body weight per day. Cyclophosphamide showed LD50 of 931.865 mg per kg body weight per day. Overall, the LD50 predicted for these compounds was higher than cyclophosphamide, except vernodalin and vernolepin. This value indicates that the South Africa Leaf sesquiterpene lactone compounds are safer than cyclophosphamide.

South Africa Leaf sesquiterpene lactone compounds have lower toxicity and are safer than cyclophosphamide. Cyclophosphamide showed higher Ames test toxicity, mutagenicity, carcinogenicity, and hepatotoxicity compared to the test compounds. The sesquiterpene lactone compounds in South Africa Leaf occur in small amounts. These compounds are known to exert additive or synergistic pharmacological effects. Botanicals (natural products) are considered to be safer with low toxicity compared to synthetic agents.[41]

Table 2: Pharmacokinetic and Toxicity Results of South Africa Leaf (*Vernonia amygdalina* Delile) Sesquiterpene Lactone Compounds and Cyclophosphamide

Property	Model Name	A	B	C	D	E	F	G
Absorption	Intestinal Absorption (human)(% Absorbed)	75.395	96.144	75.645	100.000	100.000	96.455	91.508
Absorption	Skin Permeability(log Kp)	-3.447	-3.222	-3.867	-3.118	-3.086	-2.908	-2.281
Distribution	VDss (human)(log L/kg)	-0.197	-0.236	0.017	0.107	0.156	0.198	-0.198
Distribution	Fraction Unbound (human)(Fu)	0.509	0.419	0.491	0.404	0.452	0.551	0.586
Distribution	BBB Permeability(log BB)	-0.480	-0.684	-0.193	-0.571	-0.566	-0.423	0.195
Distribution	CNS Permeability(log PS)	-3.049	-3.061	-2.975	-3.072	-3.092	-3.179	-3.055
Metabolism	CYP2D6 Substrate(Yes/No)	No	No	No	No	No	No	No
Metabolism	CYP3A4 Substrate(Yes/No)	No	Yes	No	Yes	Yes	No	No
Metabolism	CYP2D6 Inhibitior(Yes/No)	No	No	No	No	No	No	No
Metabolism	CYP3A4 Inhibitior(Yes/No)	No	No	No	No	No	No	No
Excretion	Total	0.747	0.725	0.665	1.053	1.184	1.267	0.628

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	Clearance(log ml/min/kg)							
Excretion	Renal OCT Substrate(Yes/No)	No	No	No	No	No	No	No
Toxicity	Ames Toxicity(Yes/No)	Yes	Yes	Yes	Yes	No	No	Yes
Toxicity	hERG I inhibitor(Yes/No)	No	No	No	No	No	No	No
Toxicity	hERG II inhibitor(Yes/No)	No	No	No	No	No	No	No
Toxicity	Oral Rat Acute Toxicity (LD50)(mol/kg) and (mg/kg_bw)	2.388 937.051	2.285 823.514	2.014 556.468	3.413 1243.697	3.467 1256.440	3.949 1494.301	3.569 931.865
Toxicity	Oral Rat Chronic Toxicity (LOAEL)(log mg/kg_bw/day)	1.971	1.768	1.405	1.134	1.107	2.087	0.518
Toxicity	Skin Sensitisation(Yes/No)	No	No	No	No	No	No	Yes
Toxicity	Hepatotoxicity(Yes/No)	No	No	No	No	No	No	No
Toxicity	Carcinogenicity(Yes/No)	No	No	No	Yes	No	No	Yes
Toxicity	Mutagenicity(Yes/No)	No	No	No	Yes	Yes	Yes	Yes

*A: Vernodalol; B: Vernodalin; C: Vernolepin; D: Vernomygdin; E: Vernolide; F: Hydroxyvernolide; G: Cyclophosphamide

Conclusion

In silico physicochemical, pharmacokinetic, and toxicity profiles analysis of the South Africa Leaf sesquiterpene lactone compounds showed better profiles than the standard anticancer agent, cyclophosphamide. In conclusion, hydroxyvernolide has the best physicochemical, pharmacokinetic, and toxicity profiles of the compounds investigated.

Conflict of Interest

The authors declare no conflict of interest in this study.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them

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