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IN SILICO PHARMACOKINETIC PROPERTIES OF SMALL MOLECULES FROM *VERNONIA AMYGDALINA*

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ABSTRACT

Vernonia amygdalina is a woody shrub, belonging to the family of Asteraceae. It has been used traditionally in the treatment of diabetes, microbial infestation, and malaria. The study was aimed atinvestigating the pharmacokinetic properties of small molecules from the plant using *in silico* approach. 3D structures of phytoconstituents from *Vernonia amygdalina* was retrieved from Pubchem database and Qikprop module of the Schrodinger studio was utilized to probe molecular descriptors that predict the pharmacokinetic properties and drug-likeness of these molecules in comparison to 95% of orally available drugs following their preparation with ligprep. These descriptors include; molecular weight (MW), total solvent accessible surface area (SASA), donor hydrogen bond, acceptable hydrogen bond, predicted octanol/water partition coefficient, predicted aqueous solubility, predicted apparent caco-2 cell permeability, predicted binding to human serum albumin, predicted brain/blood partition coefficient, number of likely metabolic reactions, percentage human oral absorption, Van der Waals surface area of polar nitrogen and oxygen atoms, the Lipinski's rule of five. The fraction unbound in plasma was also predicted using Watanabe model. From the results, all the compounds from *V. amygdalina* showed good aqueous solubility, good predicted human oral absorption, good predicted permeability. 87% of all the ligands studied had full compliance to the rule of five except paclitaxel, leutolin-7-glycoside and Irinotecan. 91.3% had their solvent accessible surface area within the stipulated range except paclitaxel and 5 fluorouracil. Their blood brain barrier prediction was within the range set for orally available drugs except Leutolin-7-glucorunide and Leutolin-7-glycoside. Using the Watanabe model, most of the compounds had high fraction predicted to be unbound in plasma protein. With the observed admirable predicted pharmacokinetic profile, it is promising that compounds from *Vernonia amygdalina* can be developed into therapeutic agents in the future to combat any suspected disease with which they are proven useful.

KEYWORDS: Pharmacokinetics, *in Silico*, *Vernonia amygdalina*, Schrodinger Suites.

INTRODUCTION

Vernonia amygdalina is a woody shrub, belonging to the family of Asteraceae. It is common in so many countries like; Nigeria, Singapore, Malaysia, etc. The leaves of this plant exhibit a characteristic odour and bitter taste, explaining its common English name, bitter leaf. *Vernonia amydalina* has been shown to possess diverse medicinal properties such as antimalarial,[1] anti-microbial,[2] anti-diabetic,[3] and anti-cancer effects.[4] However, small molecules from this plant have not been investigated for their drug-likeness and pharmacokinetic properties.

The highest failure rate of promising new drugs has been attributed to poor pharmacokinetic properties, thus, the need to screen potential molecules for drug-like and pharmacokinetic properties to facilitate entrance of such potential drug molecules into the market while eliminating molecules with poor profile from being subjected to expensive clinical trials.[5-7] The advancement in medical and pharmaceutical sciences has unraveled modern tools, like *in silico* ADMET prediction in drug discovery. This technique, with computational analyses, has proven to be useful for early prediction of the absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of potential drug molecules before subjecting them to the rigorous preclinical and clinical testing.[8] This modern approach in drug discovery has been successfully applied in the screening and selection of potent drugs in the treatment of diseases.[9-10]

The inclusion of pharmacokinetic considerations at earlier stages of drug discovery programs[11] using computer-based methods is becoming increasingly popular.[12] This ADMET prediction is based on molecular descriptors, which are structural or physicochemical property of a molecule or part of a

molecule.[13] The Schrodinger molecular docking studio offers an interface with which potential drug candidates can be screened for the ADMET properties via Qikprop. This module goes further to providing ranges for comparison with 95% of orally available drugs. This is particularly important as the oral route is the most convenient and non-invasive means of drug administration. These molecular descriptors include: molecular weight (MW), total solvent accessible surface area (SASA), donor hydrogen bond, acceptable hydrogen bond, predicted Caco-2 octanol/water partition coefficient, predicted aqueous solubility, predicted apparent caco-2 cell permeability, predicted brain/blood partition coefficient, number of likely metabolic reactions, percentage human oral absorption, Van der Waals surface area of polar nitrogen and oxygen atoms, the Lipinski's rule of five and the fraction of tested molecules unbound in plasma.This research was aimed at investigating the pharmacokinetic properties of phytoconstituents from *Vernonia amygdalina* using the enlisted molecular descriptors.

MATERIALS AND METHODS

The materials/softwares used in this study included: Lenovo Precision work station 6.1.7600 running Intel® Core™ i5 Duo Processor, 4.0GB RAM, 436 GB hard disk and AMD Radeon graphics card, ChemSketch professional software version 15.1, Discovery studio visualizer version 4.5 and the Schrodinger molecular docking suites version 2018-4.

Preparation of ligands

The 2-dimensional structures of the compounds (Figure 2) from *Vernonia amygdalina* were drawn using the ChemSketch professional software version 15.1 while the ligand structures used in the pharmacokinetic study were obtained from the National Centre for Biotechnology information Pubchem database (www.ncbi.nlm.nih.gov/pccompound) in SDF format and prepared with Maestro, using ligprep version 3.6, [14] an interface of the Schrodinger software suite. Accurate and high quality 3-dimensional molecular models can be generated with Ligprep from either 1-dimensional like the SMILES or from a 2-dimensional representation like the SDF presentation. Ligprep is a collection of tools designed to produce high quality, low energy 3D structure. It employed an applied force field of energy minimization with optimized potentials for liquid simulations-2005 (OPLS_2005) and filtered the ligands for computational studies. It adds hydrogen, neutralize charged groups, generate tautomers, specify chiralities or retain default chiralities, generate possible ionization states at the target pH and remove unwanted structures by desalting before generating low energy ring conformation. The output structures generated after the whole ligand preparation process was finally written to a file in Maestro format.

Determination of ADMET properties of the small molecules from *Vernonia amygdalina*

To nominate drug candidates, certain pharmacokinetics descriptors that portray their druglikeness[15] were investigated using the QikProp module of Schrodinger Suite, a program designed by Professor William L. Jorgensen.[7] In addition to predicting physically significant and pharmaceutically relevant molecular descriptors, QikProp also provides ranges for comparing predicted descriptors of each compound with those of 95% of drugs known for oral use. The analysis in the present study was run on QikProp at the normal processing mode with default settings.[16] The prepared ligands were used as input structures and their pharmacokinetics profiles with respect to properties shared by 95% of drugs known for oral use were evaluated. Compliance or deviant of the tested potential drug candidates from *V. amygdalina* to the Lipinski's rule of five was also examined before they were considered drug-like.[12]

Prediction of fraction unbound in plasma

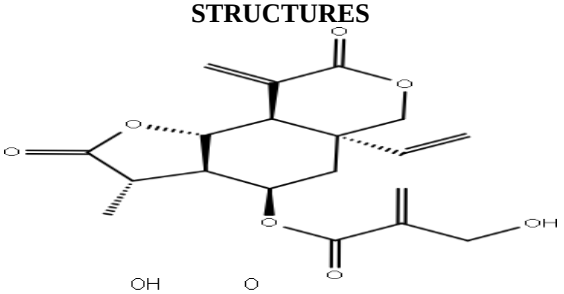
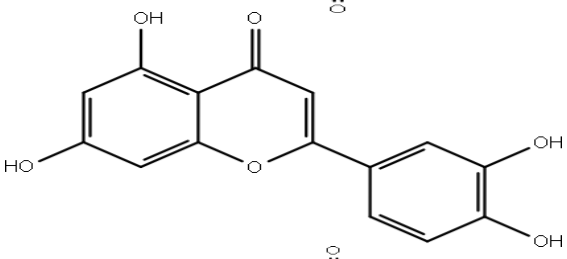
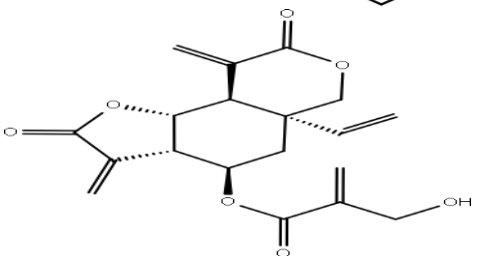
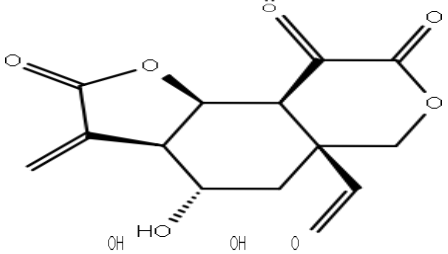
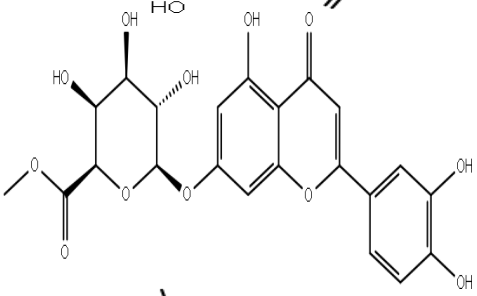
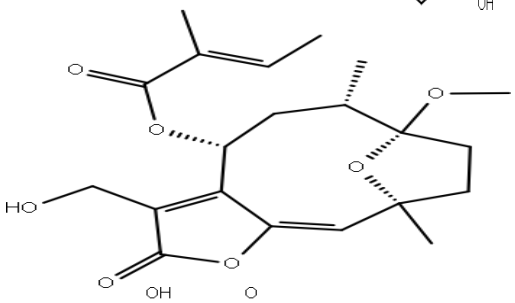
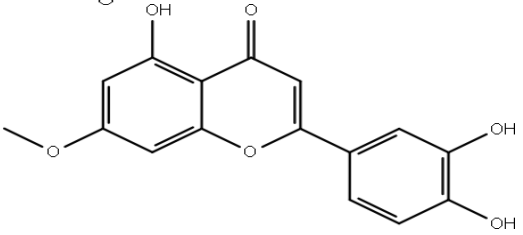
The fraction of each of the tested compounds unbound in plasma protein was predicted using the model of Watanabe *et al.*, 2018. The 3-dimensional structures of the phytochemicals from *Vernonia amygdalina* were used as input structures in the dataset.[17] The result was categorized as low (0.001-0.005), medium (0.05-0.21) and high (0.2-1.0).

RESULTS AND DISCUSSIONS

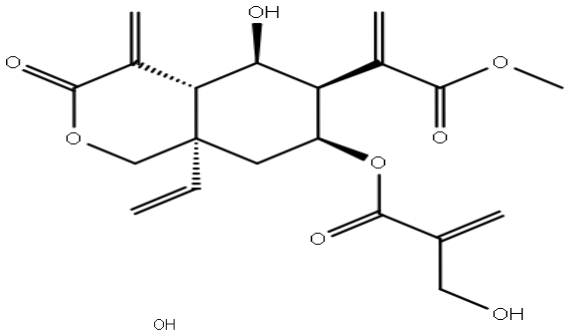
Fig. 1: Vernonia amygdalina.

Structures of the Ligands

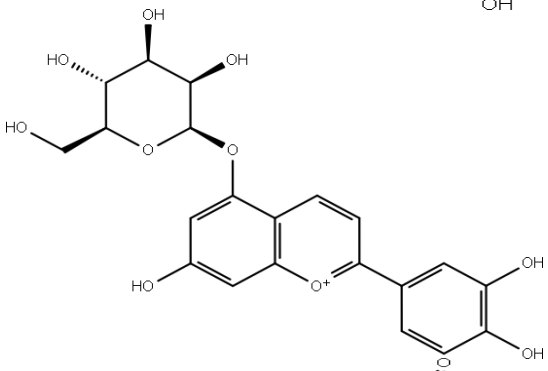
The 2-dimensional structures of each of the ligands drawn with ChemSketch professional version 15.1 are as shown below

COMPOUNDS	STRUCTURES
11,13-dihydrovernodalin	
Leutolin	
Vernodalin	
Vernolepin	
Leutolin-7-glucoronide	
Vernolide A	
Leutolin-7-methylether	

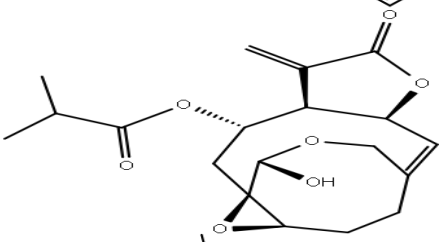
Vernodalol



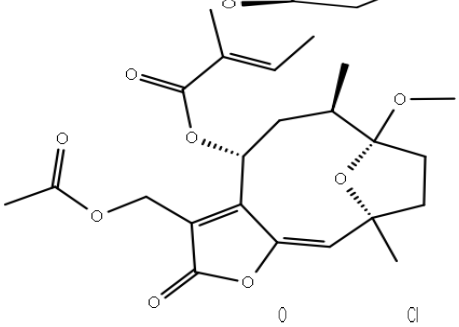
Leutolin-3-glucoside



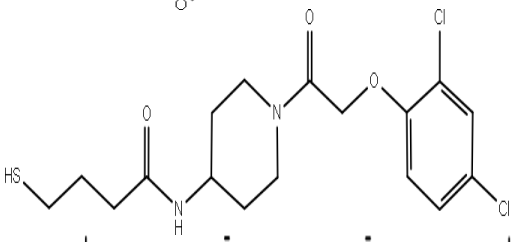
Vernomygdin



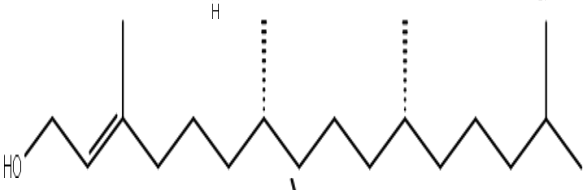
Vernolide B



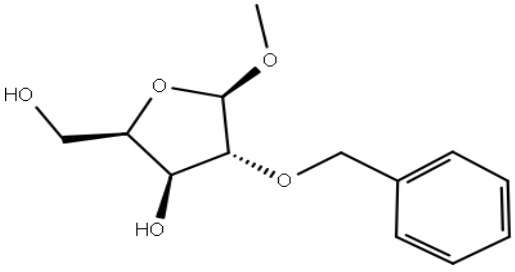
Suphanylbutamide



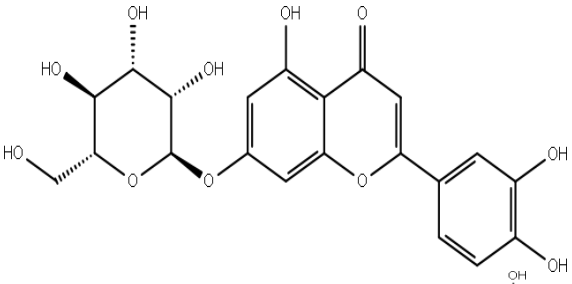
Phytol



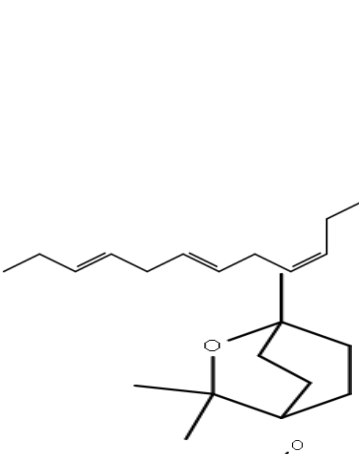
Methyl 2-0 benzylarabinofuranoside



Leutolin-7 glycosides

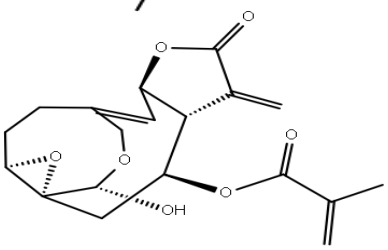


Octadecatriene

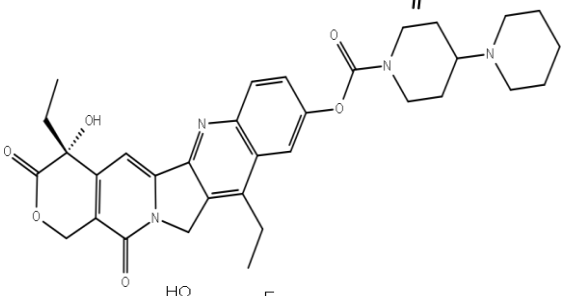


Eucalyptol

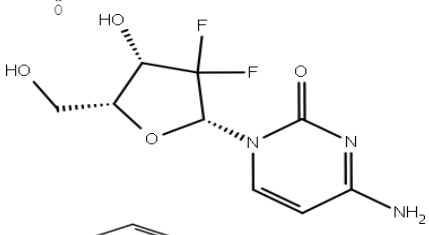
Vernolide



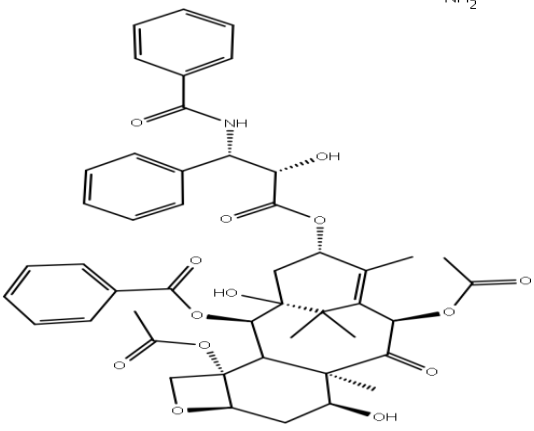
Irinotecan



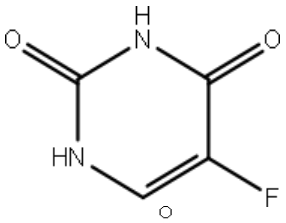
Gemcitabine



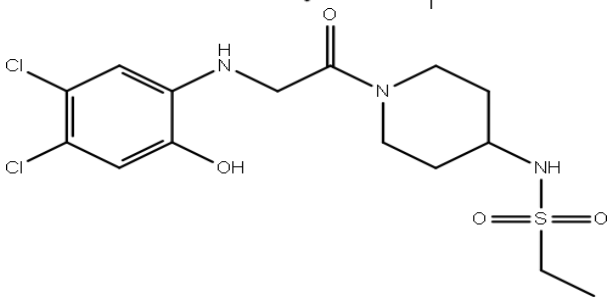
Paclitaxel



5 Fluorouracil



The native ligand (21C)



Pharmacokinetic profile of the studied compounds from *Vernonia amygdalina*

The molecular descriptors which predict pharmacokinetic profiles of the investigated compounds as investigated with the Qikprop module of the Schrodinger suite are as shown in Table 1 with the ranges for 95% of orally available drugs shown in brackets.

The molecular weight, number of hydrogen bond donor, number of hydrogen bond acceptor and octanol-water partition coefficients were used to verify the compounds adherence to Lipinski's rule of five which qualifies their drug-likeness. This Lipinski's rule of five states that an orally active drug has no more than one violation of the following criteria: molecular weight within 500 Daltons, no more than 5 hydrogen bond donor, no more than 10 hydrogen bond acceptor and an octanol-water partition coefficient not greater than 5.[12]

Table 1: Pharmacokinetic Properties of Studied ligands.

	MW (130.0-725.0)	SASA (300.0-1000.0)	Donor Hb (0.0-6.0)	Accept Hb (2.0-20.0)	QPlogPo/w (-2.0-6.5)	QplogS(-6.5-0.5)	QPPCaco <25 Poor >500 Great	QPlogBB (-3.0-1.2)	#metab (1-8)	Human Oral Absorption (>80% High <25% Low)	PSA (7.0-200.0)	Rule of Five (maximum is 4)
Vernomygdin	364.394	533.351	1	10.4	0.756	-1.775	657.967	-0.635	5	81.811	102.641	0
Vernolide_B	434.485	696.588	0	8	3.157	-4.415	538.518	-1.001	4	94.314	116.413	0
Vernolide_A	392.448	613.743	0	6.7	2.905	-3.526	636.681	-0.814	4	94.138	102.732	0
Vernolide	362.379	518.195	1	10.4	0.609	-1.545	638.876	-0.627	5	80.72	109.64	0
Vernolepin	276.288	470.775	1	7.7	0.51	-1.689	369.91	-0.877	3	75.893	97.416	0
Vernodalol	392.405	612.193	1	9.4	1.436	-2.564	142.313	-1.68	4	73.895	139.901	0
Vernodalin	360.363	569.658	0	8.7	0.953	-1.317	203.093	-1.357	3	73.83	130.317	0
Suphanylbutamide	405.338	706.486	1.8	6.75	3.232	-4.796	870.921	-0.193	4	100	70.493	0
Phytol	296.535	737.35	1	1.7	6.304	-6.85	2861.651	-0.907	3	100	22.907	1
Paclitaxel	853.918	1070.72	2	16.65	5.782	-7.068	107.425	-2.437	8	58.277	231.984	3
Octadecatriene	264.45	714.807	1	1.7	5.794	-6.43	3300.098	-0.857	5	100	22.305	1
Methyl_2_0_benzyl_ arabinofuranoside	254.282	517.346	2	8.5	0.906	-1.95	1780.877	-0.589	3	90.431	65.669	0
Leutolin	286.24	500.042	3	4.5	0.923	-3.039	42.719	-1.931	4	61.536	120.516	0
Leutolin_7_glucorunide	476.393	759.124	4	12.3	-0.02	-4.33	3.141	-4.09	7	9.81	214.26	1
Leutolin_7_methylether	300.267	526.371	2	4.5	1.778	-3.59	134.941	-1.486	4	75.481	107.086	0
Leutolin_7_glycosides	448.382	682.537	6	13	-0.976	-2.97	3.473	-3.684	7	4.989	199.713	2
Irinotecan	586.686	940.949	1	12.75	3.332	-6.454	50.844	-1.375	4	64.034	139.667	2
Gemcitabine	263.2	418.911	4	9.1	-1.176	-1.737	99.802	-1.192	3	55.841	115.314	0
Eucalyptol	154.252	377.875	0	0.75	2.467	-3.034	9906.038	0.606	1	100	7.18	0
Epivernodalol	392.405	620.155	1	9.4	1.621	-2.696	240.113	-1.503	4	79.045	136.911	0
11,13_dihydrovermodalin	362.379	613.706	0	8.7	1.091	-2.315	151.66	-1.585	3	72.366	131.597	0
5 Fluorouracil	130.078	259.644	2	3.5	-0.884	-1.02	296.526	-0.432	0	66.017	86.775	0
21Cnative_ligand 4LYF	410.315	687.155	3	9.25	1.458	-3.98	136.888	-1.374	4	73.718	114.227	0

MW=mol wt; SASA= Total solvent accessible surface area; Hb=hydrogen bond; QPlogPo/w=Predicted octanol/water partition MW=mol wt; SASA= Total solvent accessible surface area; Hb=hydrogen bond; QPlogPo/w=Predicted octanol/water partition coefficient; QPlogS=Predicted aqueous solubility; QPPCaco=Predicted apparent Caco-2 cell permeability in nm/sec; QPlogBB= Predicted brain/blood partition coefficient; #metab=Number of likely metabolic reactions; PSA=Van der Waals surface area of polar nitrogen and oxygen atoms

As earlier explained, most promising drug candidates fail to reach the market due to poor pharmacokinetic properties.[18] Today, with the help of computational techniques, these properties can be predicted before expensive clinical testing.[19] From the results above (Table 1), 87% of all the ligands studied had full compliance to the rule of five except Paclitaxel, Leutolin- 7-glycoside and Irinotecan. In this regard, Paclitaxel had a molecular weight greater than 500 Dalton, acceptable hydrogen bond greater than 10 and an octanol-water partition coefficient that was greater than 5. Leutolin-7-glycoside had its donor and accepted hydrogen bond greater than 5. Irinotecan, on the other hand, deviated in its molecular weight and acceptable hydrogen bond. However, these deviations were accommodated by Qikprop, when comparing such with attributes of 95% of orally available drugs in the market. 91.3% of all the ligands had their solvent accessible surface area (SASA) within the stipulated range except Paclitaxel and 5 Fluorouracil, whose SASA were above 1000Å and below 300Å respectively. This solvent accessible surface area predicts the movement of a molecule from aqueous solvent to a non-polar solvent such as a lipid environment. This result supports the erratic plasma values obtained when administering 5 Fluorouracil orally. Solubility, the phenomenon of dissolution of solute in solvent to give a homogenous system, is one of the important parameters to achieve desired concentration of drug in systemic circulation for any desired pharmacological response. From the results, all the compounds from *V. amygdalina* showed good aqueous solubility. However, paclitaxel, a standard drug deviated from the stipulated range. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities. 91.3% of all the studied ligands had good predicted cell permeability using the QPPCaco-2 *in silico* model except Leutolin-7-glucorunide and Leutolin-7-glycoside which showed very poor cell permeability. The Caco-2 model is the most popular and extensively characterized cell-based model employed in pharmaceutical industries and academic research fields in predicting drug permeability. 91.3% of all the studied ligands had blood brain barrier (QPlogBB) prediction within the range set for orally available drugs except Leutolin-7-glucorunide and Leutolin-7- glycoside. This indicated that both compounds would not have difficulty crossing the blood brain barrier when compared with the other compounds from *V. amygdalina.* This tendency will likely result in an adverse event from the central nervous system and it is not fitting for oral drugs. It was also observed from the result that every other compound besides leutolin-7-glucorunide and leutolin-7-glycoside had good predicted human oral absorption. Paclitaxel and leutolin-7- glucorunide showed deviation from the stipulated Van der Waal surface area (PSA).

Table 2: Predicted fraction of studied ligands unbound in plasma.

Compounds	Predicted fup (fraction unbound in plasma)	Classification*
11,13 dihydrovermodalin	0.3646	High
Epivermodalol	0.3061	High
Leuteolin-7-glycoside	0.0613	Low
Leuteolin-7-methyl ether	0.043	Low
Leuteolin-7-methyl glucuronide	0.0619	Low
Leuteolin	0.0478	Low
Leuteolinidin-3-0-glucoside	0.0723	Low
Methyl-2-0-benzyl-d-arabinofuranoside	0.4422	High
Paclitaxel	0.0428	Low
Vernodalin	0.2614	High
Vernodalol	0.3061	High
Vernolide	0.438	High
Vernolide A	0.2797	High
Vernolide B	0.1832	High
Vernomygdin	0.3993	High
21C;4LYF	0.2332	High
Tamoxifen	0.0137	Low

Table 4.3: The Model Information.

Model	Type	Output	Dataset	Accuracy
Fup Class	3 Class	Low (0.001-0.05)	Watanabe	0.676
		Medium(0.05-0.2)		
		High (0.2-1.0)		

The fraction unbound in plasma (f_{up}) is an important determinant of drug efficacy. This is because only the unbound (free) drug is capable of interacting with target proteins such as receptors, channels, ions, enzymes and

is able to diffuse between plasma and tissues for any pharmacological activity. The value of f_{up} influences renal glomerular filtration and hepatic metabolism, and consequently, it affects the volume of distribution (V_d) and the total clearance (CL_t) of a drug, which are both essential factors in pharmacokinetic studies. High f_{up} indicates more drugs available for systemic circulation and hence drugs with low f_{up} indicate lesser fraction of drugs interacting with target proteins. 58.82% of all the ligands predicted high fraction unbound in plasma while 41.18% predicted low fraction unbound in plasma. Howbeit there is room for modification for the low values predicted.

CONCLUSION

This study reveals drug-like phytochemicals from *V. amygdalina* with good physicochemical and admirable predicted pharmacokinetic properties. Since the evaluated descriptors have been reported to correlate well with *in vivo* bioavailability and are critical in developing oral dosage, it is promising that any of these phytoconstituents can be developed into therapeutic agents in the future.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Toyang N, Verpoorte R. A review of medicinal potentials of plants of the genus Vernonia (Asteraceae). *J Ethnopharmacol thnopharmacol*, 2013; 146: 681-723.
2. Yeap S, Ho W, Beh B, Liang W, Ky H. Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. *J Med plant Res*., 2010; 4: 2787-2812.
3. Reuben SI, Umar IA, James DB, Inuwa HM. Validation of the antidiabetic effects of Vernonia amygdalina delile leaf fractions in fortified diet-fed streptozotocin-treated rat model of tpe-2 diabetes. *J Diabetol*, 2017; 8: 74-85.
4. Wong FC, Woo CC, Hsu A, Tan BK. The Anti-Cancer Activities of Vernonia amygdalina Extract in Human Breast Cancer Cell Lines Are Mediated through Caspase Dependent and p53-Independent Pathways. *PLoSONE*, 2013; 8(10): 137.
5. Josh M., Pradeep S, Adarsh VK, et al. In silico evidences for the binding of phthalates onto human estrogen receptor α, β subtypes and human estrogen-related receptor γ. *Mol Simul*, 2014; 40(5): 408-417. doi:10.1080/08927022.2013.814131
6. Wang J. Comprehensive Assessment of ADMET Risks in Drug Discovery. *Curr Pharm Des.*, 2009; 15: 2195-2219.
7. Jorgensen WL, Duffy EM. Prediction of drug solubility from structure. *Adv Drug DelivRev*., 2002; 54: 355-366.
8. Stocks M, Roberts S, Ganellin R, Jefferis R. Introduction to Biological and Small Molecule Drug Research and Development Theory and case studies. *Elsevier*. Publishedonline, 2013.
9. López-Vallejo F, Caulfield T, Martínez-Mayorga K, et al. Integrating virtual screening and combinatorial chemistry for accelerated drug discovery. *Comb Chem HighThroughput Screen*, 2011; 14: 475-487.
10. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. *Int J Mol Sci.*, 2019; 20(18): 4331.
11. Hodgson J. ADMET – turning chemicals into drugs. *Nat Biotechnol*, 2001; 19: 722-726.
12. Lipinski C., Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*., 2001; 46: 3-26.
13. Ntie-Kang F. An in silico evaluation of the ADMET profile of the StreptomeDB database. *Springerplus*, 2013; 2(Hodgson 2001): 353. doi:10.1186/2193-1801-2-353.
14. LigPrep. version 3.6, Schrödinger, LLC, New York, NY. Published online, 2015.
15. Ekins S, Waller C, Swaan P, Cruciai G, Wrigton S, Wikel J. Progress in predicting human ADME parameters in silico. *J Pharmacol Toxicol Methods*, 2000; 44(1): 251-272.
16. QikProp. Version 4.6. *Schrödinger, LLC, New York.* Published online, 2015.
17. Watanabe R, Esaki T, Kawashima H, Natsume-Kitatani, Y. Nagao C, Ohashi R, Mizuguchi K. Predicting fraction unbound in human plasma from chemical structure: improved accuracy in the low value ranges. *Mol Pharmacol*, 2018; 15(11): 5302-5311.
18. Ntie-Kang F, Mbah JA, Lifongo LL, et al. In silico drug metabolism and pharmacokinetic profiles of natural products from medicinal plants in the Congo basin. *Silico Pharmacol*. Published online, 2013; 1-12.
19. Etti I, Abdullah R, Hashim NM, et al. Artonin E and structural analogs from Artocarpus species abrogates estrogen receptor signaling in breast cancer. *Molecules*, 2016; 21(7). doi:10.3390/molecules21070839