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RESEARCH ARTICLE

Investigating the Anti-Venom Potential of Dolichos Trilobus Root Extract: A Computational and Cheminformatics Approach

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ABSTRACT

Snake-bite has remained an enduring medical problem for many decades and little progress has been made in reducing mortality. In Nigeria, snake bites remain a common and serious problem especially in rural areas where access to prompt and effective treatment is limited. The tropical climate and the favorable environmental factors within this region are known to provide suitable habitat for snakes. The society is largely agrarian and the greater part of its population engaged in farming activities, livestock rearing, hunting and collection of firewood. These activities constitute occupational hazard or snake bites and are responsible for sustaining the high burden of the problem. The root of *Dolichos trilobus* was air dried, pulverized to powder and extracted with 80.00 % methanol (v/v). The crude extract was fractionated by liquid-liquid extraction into methanol, ethyl acetate and hexane fractions. Phytochemical analysis was carried out on the crude extracts and the fractions which revealed the presence of alkaloids, terpenes, flavonoids, carbohydrates, anthraquinones, cardiac glycosides, steroids, tannins and saponins. The methanol fraction was subjected to GC-MS analyses and 4-(3,4-Dimethoxyphenyl)-6-phenylpyrimidin-2-ol, 3-Benzylamino-5,6-diphenyl-1,2,4-triazine and 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine were detected.

Keywords- : Anti-venom; Plants; Natural Product; Phytochemicals; SwissADME

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INTRODUCTION

Snake-bite poisoning is an important unheeded disease in most of the developing countries. Viper snakes are among the most common types of venomous snakes which are responsible for many envenoming and deaths in most tropical areas. African countries are global diversity center for feared snake families, *Vipers, Krait* and *Cobras*. The risk of snake-bite to people in the rural region of tropical countries, where most people engage in agricultural, pastoral, and other outdoor livelihoods is moderate to high.[1]

Serum based anti-venom treatment is very expensive, out of reach for the common man and has several adverse effects because of the foreign proteins while herbal treatment is cheap, easily available, stable at room temperature and could neutralize a wide range of venom antigen without side effects.[1] The use of plants against the effects of snake bite has long been recognized, even in modern times but only for the last twenty years has it merited closer scientific attention.[2]

Many reports from the world over have mentioned plants reputed to neutralize the action of snake venoms, but only a few attribute such activity to certain chemical compounds identified in them[3] and even less are concerned with a possible mechanism of action.[4] Here, the use of computational and cheminformatics methods cannot be over stated or overemphasized. The prediction of the potential biological and pharmacological activity of chemical compounds identified in plants known to possess anti-snake venom activity will go a long way in advancing the quest to find plant based anti-venom treatments.

Dolichos tribolus plant, called 'katala' in Fulfulde belongs to the plant family *Fabaceae* (the pea family). According to Mr. Danladi Adamu (personal communication), a renowned traditional herbalist in Bauchi, Bauchi State, Nigeria, 'katala' has been used for a long time by herbalists for both curative and preventive purposes against snake-bite envenomation and snake bites. According to the herbalist, the herb has been used to cure victims of viper and cobra envenomation.

Figure 1: Root of Dolichos tribolus

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For curative purposes, the infusion an index finger size of the herb is administered orally to the patient while for preventive purposes, the dried and pulverized herb is taken with milk or the fresh herb is chewed more than twice with potash.

MATERIALS AND METHODS

Dolichos tribolus root tuber was collected from Alkaleri Local Government Area, Bauchi State and was identified by Dr. Usman H. Dukku of the Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi. The sample was washed free of any sand particles or foreign materials, diced into smaller pieces using a clean knife and air dried for three weeks. The dried sample was pulverized to fine powder using a wooden pestle and mortar and stored in a transparent plastic container for further investigations.

The pulverized sample (600.00 g) was weighed using a weighing balance and transferred into 3000.00 cm^3 round bottomed flask and extracted cold with 80.00 % methanol for 72 hours. The extract was collected into five 1000 cm^3 beakers by sieving and washing with 2000.00 cm^3 of 80.00 % methanol. The crude extract was concentrated using a Stuart Scientific AG rotary evaporator and subsequently transferred into a weighed 1000 cm^3 beaker and placed in a drying cabinet at 38 °C for 48 hours. The beaker containing the dried crude extract was weighed and the weight recorded.

Liquid-liquid extraction as described by Harborne (1998)[5] was used to fractionate the crude extract into hexane, ethyl acetate and methanol fractions. 30.00 g of crude extract was weighed into a separating funnel and dissolved with 200.00 cm^3 of 80 % methanol. 200.00 cm^3 of triple distilled n-hexane was measured and added into the separating funnel. The solution was thoroughly mixed and allowed to stand for 24 hours. The hexane layer was collected in a 200.00 cm^3 round bottomed flask. The same procedure was repeated at 3 hour intervals until a clear hexane layer was observed. The 80.00 % methanol layer was air dried and repacked into a clean separating funnel and treated with ethyl acetate as with hexane above. The same procedure was employed in treatment with absolute ethanol. All fraction solutions were evaporated using a rotary evaporator and subsequently transferred into three different weighed 100 cm^3 beakers and concentrated to dryness on a water bath. The beakers containing the dried fractions were all weighed and the weights recorded.

Phytochemical screening of the powder, crude extract and fractions was carried out using standard methods described by Harborne (1998)[5].

Gas chromatographic-mass spectrophotometric (GC-MS) analysis was carried out on the methanol fraction using Agilent Technology 7890B GC System coupled with Agilent Technology 5977A MSD epuipment.

Three compounds detected by the GC-MS analysis were analized with SwissADME to predict their absorption, distribution, metabolism and excretion (ADME) parameters, physicochemical properties, pharmacokinetic descriptors, drug-likeness, medicinal chemistry potentials. Biological target and similarity predictions were also determined using the SwissADME web tool.

RESULTS AND DISCUSSION

The percent recovery for each fraction shows that the hexane fraction (0.20 g) was the least obtained while the methanol fraction (18.70 g) was highest.The percent recovery for the crude extract of *Dolichos tribolus* root (Table 1) compares favorably with the methanol extract of *Abutilon indicum* where 4.37 g was obtained from the

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same amount of dried powdered sample of the plant.[6]

Table 1: Percent Recovery of Crude Extract and Fractions

	IW (g)	FW (g)	IW - FW (g)	PR (%)
CE	600.00	541.00	58.40	9.70
HF	30.00	29.80	0.20	0.60
EAF	29.80	1.90	1.90	6.30
MF	27.90	11.30	18.70	67.03

KEY: IW = Initial Weight; FW = Final Weight; PR = Percent Recovery; CE = Crude Extract; HF = Hexane Fraction; EAF = Ethyl Acetate Fraction; MF = Methanol Fraction

Phytochemical Screening

Phytochemical screening of dried whole powder, crude extract, hexane fraction, ethyl acetate fraction and methanol fraction of *Dolichos tribolus* root (Table 2) revealed the presence of all phytochemicals tested for with the exception of terpenes which tested negative in all plant samples. In addition, alkaloids, saponins, tannins and carbohydrates were absent in the hexane fraction while the ethyl acetate extract tested negative for alkaloids, tannins and carbohydrates. Also, the experiment showed marked similarity in the phytoconstituents of the dried whole powder, crude extract and methanol fraction of the plant where only the methanol fraction of *Dolichos tribolus* root is (with the exception of steroids and terpenes) identical with the phytochemical constituents of the methanolic extract of the stem bark of *Neocarya macrophylla*[8] which demonstrated significant antivenin activity against *Naja nigricollis* venom.

steroids was absent in the methanol fraction. Cardiac glycosides tested positive in all tested samples which is dissimilar to the phytochemical profile of *Tridax procumbens* (found to have effect on the clotting time of human blood) where the aqueous, hydro-alcoholic and petroleum ether extracts all tested negative for glycosides.[7] However, the phytochemical profile of the hydro-alcoholic extract of the same plant is similar to that of the methanol fraction of *Dolichos tribolus* where alkaloids, tannins, flavonoids, carbohydrates, anthraquinones and glycosides were all detected in the extracts of both plants. Also, as shown by this study, the phytochemical constituents of

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Table 2: Phytochemical Screening of Whole Powder, Crude Extract, Ethyl Acetate fraction and methanol fraction of Dolichos tribolus

CONSTITUENTS	WHOLE POWDER	CRUDE EXTRACT	HEXANE FRACTION	ETHYL ACETATE FRACTION	METHANOL FRACTION
ALKALOIDS	++	+++	-	-	+++
SAPONINS	+	++	-	+	+ (***TRACES***)
TANNINS	+	++	-	-	++
FLAVONOIDS	+++	+++	+	+ (***TRACES***)	+++
CARBOHYDRATES	+++	++	-	-	++
STEROIDS	++	+++	+++	++	-
TERPENES	-	-	-	-	-
ANTHRAQUINONES	++	+	+	++	+
CARDIAC GLYCOSIDES	+++	+++	+++	++	+++

KEY: '+' = Presence of phytoconstituent; '-' = Absence of phytoconstituents

Similarly, the phytochemical profile of the ethyl acetate and methanol fractions of *Dolichos tribolus* root is close to that of the ethyl acetate and methanol extracts of *Azima tetracantha* Lam. leaves found to be active against different ezymes of venoms from *Bungarus caeruleus* and *Vipera russelli*.[9]

Gas Chromatographic-Mass Spectral Analysis of Methanol Fraction

The medicinal use of 4-(3,4-Dimethoxyphenyl)-6-phenylpyrimidin-2-ol has not been reported in the literature but the medicinal importance of pyrimidine based compounds has been established.[10] Some structurally similar 2 amino- 4, 6 diaryl substituted pyrimidines were synthesized and then screened for antibacterial and herbicidal activity and it was found that they exhibited good antibacterial activities.[11]

Although medicinal activity of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine has not been reported in the literature, Coumarin and its Umbelliferone derivatives were found to provide 40 % protection against snake venom activity.[2]

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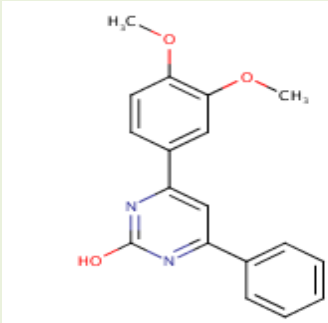
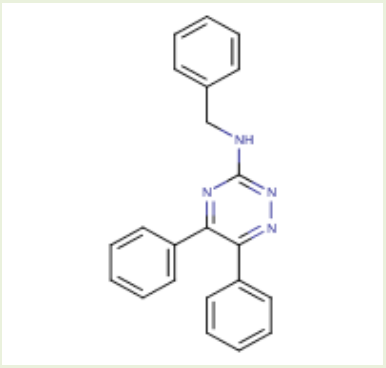
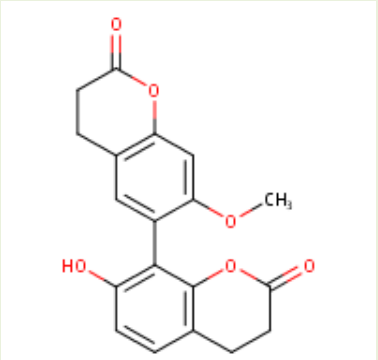
Table 3: Gas Chromatographic and Mass Spectral Data of Methanol Fraction

Name of Component	Structure	m/z	Abundance	RT (min)	NS (%)
4-(3,4-Dimethoxyphenyl)-6-phenylpyrimidin-2-ol:		116.00	24970.23	16.98	72.96
		158.00	82930.37		
		161.00	24637.50		
		162.00	113195.87		
		171.00	43203.18		
		265.00	27085.50		
		291.00	43952.00		
		307.00	64016.00		
		308.00	418880.00		
		309.00	86072.00		
3-Benzylamino-5,6-diphenyl-1,2,4-triazine		77.00	90136.09	17.93	69.23
		105.00	66876.32		
		133.00	545708.44		
		135.00	82874.09		
		163.00	383055.88		
		165.00	173229.09		
		178.00	2424501.25		
		179.00	276802.19		
		338.00	844608.00		
		339.00	181568.00		
6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine		152.30	76504.00	16.98	72.96
		161.00	79477.50		
		175.00	54545.86		
		176.00	38241.23		
		207.00	37108.41		
		291.00	30800.00		
		305.00	242240.00		
		306.00	50152.00		
		336.00	286656.00		
		337.00	61432.00		

Key: RT = Retention Time; NS = NIST Score

Coumarin, the simplest representative of its class occurs often in considerable amounts in anti-snake venom plants where Herniarin (7-methoxy-coumarin) and ayapin (6,7-methylenedioxy-coumarin), isolated from the Amazonian anti-snake venom plant Eupatorium triplinerve, were shown to exhibit considerable hemostatic activity.[12] The mass spectra and of the detected components are shown on Appendix 2.

Computational and Cheminformatics Predictions

The four components detected by GC-MS analysis were analyzed with SwissADME.ch, an online computational and cheminformatics software to determine their physicochemical properties, solubility

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properties, pharmacokinetic parameters, drug likeness, target predictions and similarity predictions.

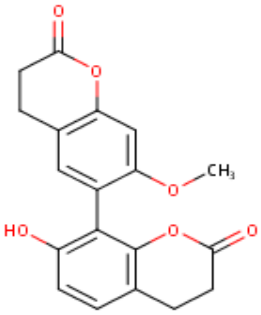
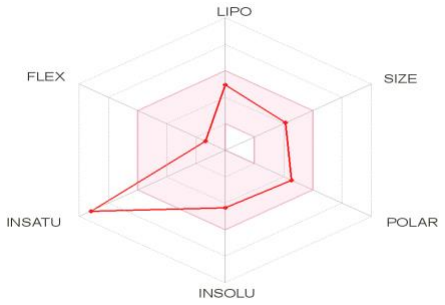
Bioavailability

Table 4 shows the two-dimensional chemical structure of the query molecules expressing the form from which the predictions were computed.[13] The bioavailability radar expresses, at first glance, the drug-likeness of the molecules taking into account six physicochemical properties: lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), flexibility (FLEX) and saturation (INSATU). Each range on their respective axis was defined by descriptors and expressed as a pink area under which the plot of each molecule has to fall entirely to be considered drug-like.[13,14,15] The results of the analysis show that all parameters fall within the drug-like area with the exception of saturation which was low for all three compounds.

Physicochemical Parameters

Table 5 shows the physicochemical properties of the detected components. The values of the physicochemical parameters were computed with the 2.3.0 version of 'OpenBabel' computer program.[16] According to this model, a compound with suitable physicochemical parameters should not have the fraction of carbons in the sp3 hybridization below 0.25, and not more than nine rotatable bonds.[13] The detected components all have less than nine rotatable bonds and less than 0.25 fraction of sp3 carbons with the exception of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine which has a Csp3 of 0.26. The Polar Surface Area (PSA) is calculated using the fragmental technique called Topological Polar Surface Area (TPSA) defined as the summation of tabulated surface contributions of polar atoms (also taking into account their bonding patterns) while considering sulfur and phosphorus as polar atoms.[16]

Table 4: Two-dimensional Structure and Bioavailability Radar of Detected Components

ENTRY	Two-dimensional Structure	Bioavailability Radar
6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine		

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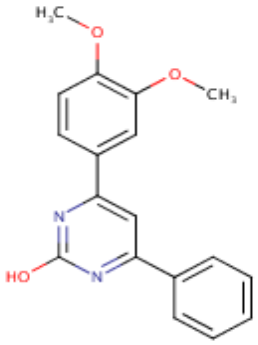
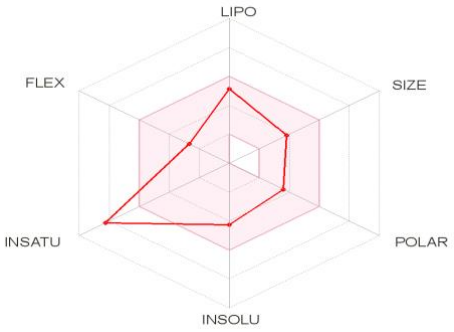
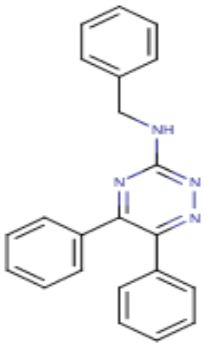
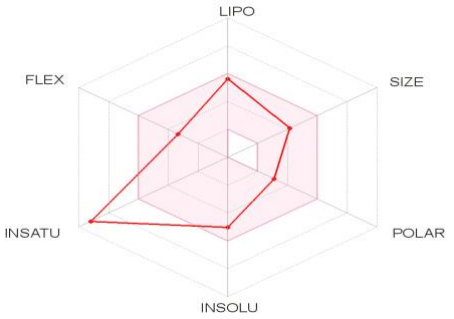
6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol





3-Benzylamino-5,6-diphenyl-1,2,4-triazine





There is an inverse relationship between PSA and Human Intestinal Absorption (HIA) and thus with cell wall permeability.[17] The model stipulates that a compound with optimum cell permeability should possess a TPSA < 140 Å².[13] All detected components have TPSA less than 140 Å² with 3-Benzylamino-5,6-diphenyl-1,2,4-triazine having the lowest TPSA (50.70 Å²) and therefore the highest cell permeability while 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine has the highest TPSA (82.06 Å²) and thus is the least cell permeant.

Table 5: Physicochemical Properties of Detected Components

ENTRY	6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine	6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol	3-Benzylamino-5,6-diphenyl-1,2,4-triazine
Molecular Weight	340.33 g/mol	308.33 g/mol	338.41 g/mol
Number of heavy atoms	25	23	26
Number of aromatic heavy atoms	12	18	24
Fraction Csp3	0.26	0.11	0.05
Number of rotatable	2	4	5

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bonds			
Number of H-bond acceptors	6	5	3
Number of H-bond donors	1	1	1
Molar Refractivity	89.1	87.91	104.49
TPSA (Å²)	82.06	64.47	50.70
Consensus *Log P*o/w	2.86	3.05	4.09

Key: Csp3 = sp3 Carbons; TPSA = Total Polar Surface Area

The Partition Coefficient between *n*-octanol and water (log *P*o/w) is the classical descriptor for Lipophilicity and hence holds critical importance to the physicochemical property for pharmacokinetic drug discovery.[18] Here, multiple predictors are used either to select the most accurate method for a given chemical series or to generate consensus estimation.[19] SwissADME utilizes five predictive models (XLOGP3, WLOGP, MLOGP, SILICOS-IT and iLOGP) and the consensus log *P*o/w is the arithmetic mean of the values predicted by the five proposed methods.[13] All three compounds analyzed were all lipophilic with 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine (2.86) being least lipophilic while 3-Benzylamino-5,6-diphenyl-1,2,4-triazine (4.09) is most lipophilic.

Pharmacokinetic Descriptors

Table 6 shows the pharmacokinetic parameters of the components which are the individual ADME behaviors of the molecules. Multiple Linear Regression model was used to predict the skin permeability coefficient (*K*p).[20] The more negative the log *K*p (cm/s), the less skin permeant the molecule. The predictive model returned log *K*p values of -6.53 cm/s, -5.73 cm/s and -5.29 cm/s for 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine, 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol and 3-Benzylamino-5,6-diphenyl-1,2,4-triazine respectively. This means 3-Benzylamino-5,6-diphenyl-1,2,4-triazine is predicted to be the most skin permeant while 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine is the least skin permeant.

Transport proteins ensure the uptake of essential nutrients including glucose, amino acids, vitamins, and nucleosides that otherwise would not passively diffuse into the brain.[21] Since the Blood-Brain Barrier (BBB) capillary network is particularly discriminatory with respect to passive diffusion of molecules, it represents a major hurdle to be overcome when attempting to access targets situated within the Central Nervous System (CNS).[22] This makes the BBB permeability of compounds a very important factor in drug discovery and design. In SwissADME, the predictions for passive human gastrointestinal absorption (HIA) and BBB permeation both consist in the readout of the BOILED-Egg model17, an intuitive graphical classification model.[13]

The BBB permeant query for a potential drug should return a ‘yes’ and in this regard, only 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine returned a ‘No’ which means it possesses no capacity to permeant through the BBB into the CNS. However, both 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol and 3-Benzylamino-5,6-diphenyl-1,2,4-triazine were predicted BBB permeant and can therefore be absorbed into the CNS. The knowledge about compounds being substrate or non-substrate of the Permeability Glycoprotein

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(P-gp) is key to appraise active efflux through biological membranes, for instance from the gastrointestinal wall to the lumen or from the brain.[23] One major role of P-gp is to protect the central nervous system (CNS) from xenobiotics[24] and the interaction of molecules with Cytochromes P450 (CYP) is also very important in the drug discovery quest. It has been suggested that CYP and P-gp can process small molecules synergistically to improve protection of tissues and organisms.[25] It was also estimated that 90.00 % of therapeutic molecules are substrates of five major isoforms, namely: CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4.[26] Inhibition of these isoenzymes is a major cause of pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites.[27,28]

Table 6: Pharmacokinetic, Drug-Likeness and Medicinal Chemistry Parameters of Detected Components

ENRTY	SUB-ENTRY	6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine	6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol	3-Benzylamino-5,6-diphenyl-1,2,4-triazine
GI Absorption		High	High	High
BBB Permeant		No	Yes	Yes
P-gp Substrate		Yes	No	Yes
CYP1A2 inhibitor		Yes	Yes	Yes
CYP2C19 inhibitor		Yes	Yes	Yes
CYP2C9 inhibitor		Yes	Yes	Yes
CYP2D6 inhibitor		Yes	Yes	Yes
CYP3A4 inhibitor		No	Yes	Yes
***Log K_p* (Skin Permeation)**		-6.53 cm/s	-5.73 cm/s	-5.29 cm/s
Drug-Likeness	**Lipinski**	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
	Ghose	Yes	Yes	Yes
	Veber	Yes	Yes	Yes
	Egan	Yes	Yes	Yes
	Muegge	Yes	Yes	Yes
	Bioavailability Score	0.55	0.55	0.55
Medicinal Chemistry	**PAINS**	0 alert	0 alert	0 alert
	Brenk	1 alert: Phenol ester	0 alert	0 alert
	Lead-Likeness	Yes	Yes	No; 1 violation: XLOGP3>3.5
	Synthetic accessibility	2.98	2.52	3.17

Key: BBB = Blood Brain Barrier; CYP =Cytochromes P450; P-gp = Permeability Glycoprotein; GI = Gastro Intestinal

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It is therefore of great importance for drug discovery to predict the propensity with which the molecule will cause significant drug interactions through inhibition of CYPs, and to determine which isoforms are affected and SwissADME enables the estimation of a chemical as a substrate of P-gp or inhibitor of the most important CYP isoenzymes. The models return "Yes" or "No" if the molecule under investigation has higher probability to be substrate or non-substrate of P-gp and inhibitor or non-inhibitor of a given CYP (Antoine *et al* 2017). From the results of the ADME analysis obtained (Table 6), it is seen that only 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol is a non P-pg substrate while all three compounds were predicted to inhibit all CYPs with the exception of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine which returned a 'No' for inhibition of CYP3A4.

Drug-Likeness

Drug-likeness estimates qualitatively, with respect to bioavailability, the chances of a molecule becoming an oral drug and is established from structural or physicochemical inspections of development compounds advanced enough to be considered oral drug-candidates.[13] This SwissADME section gives access to five different rule-based filters, with diverse ranges of properties inside of which the molecule is defined as drug-like.[13] All three compounds analyzed were predicted drug-like by all the filters with all three compounds having a bioavailability score of 0.55.

Medicinal Chemistry

The medicinal chemistry section supports medicinal chemists in their daily drug discovery endeavors. PAINS (pan assay interference compounds, a.k.a. frequent hitters or promiscuous compounds) are molecules containing groups showing potent response in many assays irrespective of the protein target.[13] 481 recurrent fragments considered as potentially leading to promiscuous compounds were identified and and SwissADME, computing from this filter, returns warnings if such moieties are found in a molecule under evaluation.[29] In the same vein, 105 fragments which are putatively toxic, chemically reactive, metabolically unstable or bear properties responsible for poor pharmacokinetics were identified.[30] All queries for PAINS and Brenk returned positive with the exception of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine which returned an alert defined as 'phenol ester' indicating the possible toxicity of phenol ester fragments in predicted drug body relationships.

Synthetic Accessibility (SA) entails the analysis of more than 13 million compounds immediately deliverable by vendors and the most frequent molecular fragments in this large collection indicates a probably high SA, while rare fragments imply a difficult synthesis.[31] After normalization, the SA Score ranges from 1.00 (very easy) to 10.00 (very difficult).[13] From the results obtained, it is seen that all detected compounds are predicted as easily synthesized with 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol being most readily synthesized while 3-Benzylamino-5,6-diphenyl-1,2,4-triazine is predicted most difficult.

Similarity Predictions

SwissSimilarity is a simple yet powerful tool for the rapid screening of small to very large libraries of drugs, bioactive small molecules and commercially available or virtual, yet synthesizable, compounds.[32]

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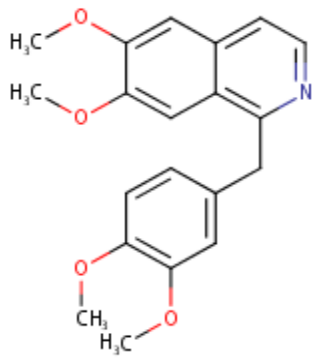
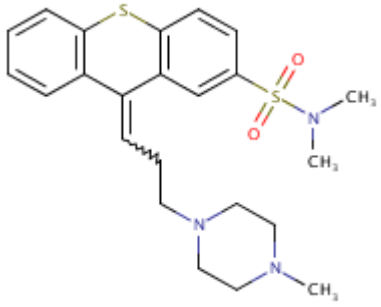
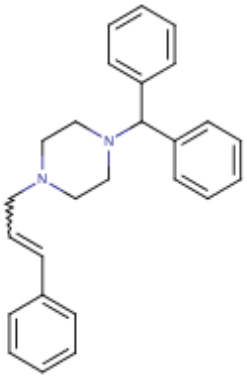
Table 7: Similarity Predictions of Detected Components

6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine	6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol	3-Benzylamino-5,6-diphenyl-1,2,4-triazine
		
Papaverine	Thiothixene	Cinnarizine

Direct *in silico* screening is performed using different and complementary two-dimensional and three-dimensional approaches to support hit findings by selecting compounds or enriching chemical collections with new molecules similar to known active ones.[33] The similarity prediction for 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine returned Papaverine (Table 7) which is a drug approved for the treatment of spasms of the gastrointestinal tract by acting as a vasodilator that relaxes smooth muscles in the cardiovascular system to help them dilate thereby lowering blood pressure and enhancing free flow of blood.[34] 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol was predicted similar to Thiothixene (Table 7) which is a typical antipsychotic drug of the Thioxanthene class which is related to Chlorprothixene and is used in the treatment of psychoses like schizophrenia and bipolar mania.[35] Cinnarizine (Table 7) was predicted to be similar to 3-Benzylamino-5,6-diphenyl-1,2,4-triazine. Cinnarizine is an antihistamine and calcium channel blocker of the diphenylmethylpiperazine group and is also known to promote cerebral blood flow used to treat cerebral apoplexy and cerebral arteriosclerosis.[36]

Target Precictions

SwissTargetPrediction is a web tool which utilizes both two-dimensional and three-dimensional similarity criteria through dual-scoring logistic regression to predict the most likely protein targets of bioactive molecules.[33] Table 4.9, Table 4.10 and Table 4.11 show the target predictions of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine, 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol and 3-Benzylamino-5,6-diphenyl-1,2,4-triazine respectively.

One of the predicted targets of 3-Benzylamino-5,6-diphenyl-1,2,4-triazine is Acetylcholinesterase (Uniprot ID: P22303). This suggests that the compound can act as

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an inhibitor of the kinase protein thereby compensating for the death of cholinergic neurons and offer symptomatic relief by inhibiting acetylcholine (ACh) turnover and restoring synaptic levels of this neurotransmitter.[37]

Serine/threonine-protein kinase (Uniprot ID: P42345) was predicted as a target site for 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine. The synthesis of a variety of serine proteinases capable of affecting the haemostatic system as they act on macromolecular substrates of the coagulation, fibrinolytic, and kallikrein-kinin systems, and on platelets to cause an imbalance of the haemostatic system of the prey was shown.[38] This confers an anti-snake venom potentiality on 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine with respect to its possible inhibition of serine proteinase which is an active component of snake venom.

LDL-associated phospholipase A2 (Uniprot ID: Q13093) is a predicted target site for 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol. Phospholipases A2 are abundant in snake venoms and besides playing a digestive role in phospholipid hydrolysis, they also exert a wide range of pharmacological activities such as neurotoxicity, myotoxicity, edema-inducing activity.[39]

Table 8: Target Prediction of 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine.

Target	Common name	Uniprot ID	ChEMBL ID	Target Class
Monoamine oxidase B	MAOB	P27338	CHEMBL2039	Oxidoreductase
Macrophage migration inhibitory factor	MIF	P14174	CHEMBL2085	Enzyme
Presequence protease, mitochondrial	PITRM1	Q5JRX3	CHEMBL3124731	Enzyme
Estrogen receptor alpha	ESR1	P03372	CHEMBL206	Nuclear receptor
Estrogen receptor beta	ESR2	Q92731	CHEMBL242	Nuclear receptor
PI3-kinase p110-delta subunit	PIK3CD	O00329	CHEMBL3130	Enzyme
Beta-glucuronidase	GUSB	P08236	CHEMBL2728	Enzyme
Hepatocyte growth factor receptor	MET	P08581	CHEMBL3717	Kinase
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450
Tyrosine-protein kinase ABL	ABL1	P00519	CHEMBL1862	Kinase
Vascular endothelial growth factor receptor 2	KDR	P35968	CHEMBL279	Kinase
Serine/threonine-protein kinase	MTOR	P42345	CHEMBL2842	Kinase

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PI3-kinase p110-beta subunit	PIK3CB	P42338	CHEMBL3145	Enzyme

Key: ChEMBL = Chemical Data Base of Bioactive Drug-like Small Molecules; Uniprot = Universal Protein Resource

The predicted reactivity of (3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol with LDL-associated phospholipase A2 and the possible inhibition of serine proteinase by 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine is therefore an important indicator of the anti-venom potential of *Dolichos tribolus* root.

Table 9: Target Predictions of 6-(3,4-Dimethoxyphenyl)-4-Phenylpyrimidin-2-ol

Target	Common name	Uniprot ID	ChEMBL ID	Target Class
Adenosine A1 receptor	ADORA1	P30542	CHEMBL226	Family A G protein-coupled receptor
Adenosine A2a receptor	ADORA2A	P29274	CHEMBL251	Family A G protein-coupled receptor
Monoamine oxidase B	MAOB	P27338	CHEMBL2039	Oxidoreductase
5-lipoxygenase activating protein	ALOX5AP	P20292	CHEMBL4550	Other cytosolic protein
GABA receptor alpha-3 subunit	GABRA3	P34903	CHEMBL3026	Ligand-gated ion channel
GABA receptor alpha-1 subunit	GABRA1	P14867	CHEMBL1962	Ligand-gated ion channel
GABA receptor alpha-2 subunit	GABRA2	P47869	CHEMBL4956	Ligand-gated ion channel
Maltase-glucoamylase	MGAM	O43451	CHEMBL2074	Hydrolase
Hypoxia-inducible factor 1 alpha	HIF1A	Q16665	CHEMBL4261	Transcription factor
Beta-secretase 1	BACE1	P56817	CHEMBL4822	Protease
LDL-associated phospholipase A2	PLA2G7	Q13093	CHEMBL3514	Enzyme
Muscle glycogen phosphorylase	PYGM	P11217	CHEMBL3526	Enzyme
Adenosine A3 receptor	ADORA3	P0DMS8	CHEMBL256	Family A G protein-coupled receptor

Key: ChEMBL = Chemical Data Base of Bioactive Drug-like Small Molecules; Uniprot = Universal Protein Resource

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Table 10: Target Predictions of 3-Benzylamino-5,6-diphenyl-1,2,4-triazine

Target	Common Name	Uniprot ID	ChEMBL ID	Target Class
Adenosine A1 receptor	ADORA1	P30542	CHEMBL226	Family A G protein-coupled receptor
Adenosine A2a receptor	ADORA2A	P29274	CHEMBL251	Family A G protein-coupled receptor
Cannabinoid receptor 2	CNR2	P34972	CHEMBL253	Family A G protein-coupled receptor
Cathepsin L	CTSL	P07711	CHEMBL3837	Protease
Cannabinoid receptor 1	CNR1	P21554	CHEMBL218	Family A G protein-coupled receptor
Adenosine A2b receptor	ADORA2B	P29275	CHEMBL255	Family A G protein-coupled receptor
Acetylcholinesterase *(by homology)*	ACHE	P22303	CHEMBL220	Hydrolase
Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor
Glucocorticoid receptor	NR3C1	P04150	CHEMBL2034	Nuclear receptor
Progesterone receptor	PGR	P06401	CHEMBL208	Nuclear receptor

Key: ChEMBL = Chemical Data Base of Bioactive Drug-like Small Molecules; Uniprot = Universal Protein Resource

CONCLUSION

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Also, 6-(7-hydroxycoumarin-8-yl)-7-methoxycoumarine and 3-Benzylamino-5,6-diphenyl-1,2,4-triazine detected in the methanol fraction showed marked promise, computationally towards anti-venom activity by having potential capacity to react with venom Serine Proteinase and Phospholipase A2. This also supports the herbal assertion that the root of *Dolichos tribolus* has anti-venom potential.

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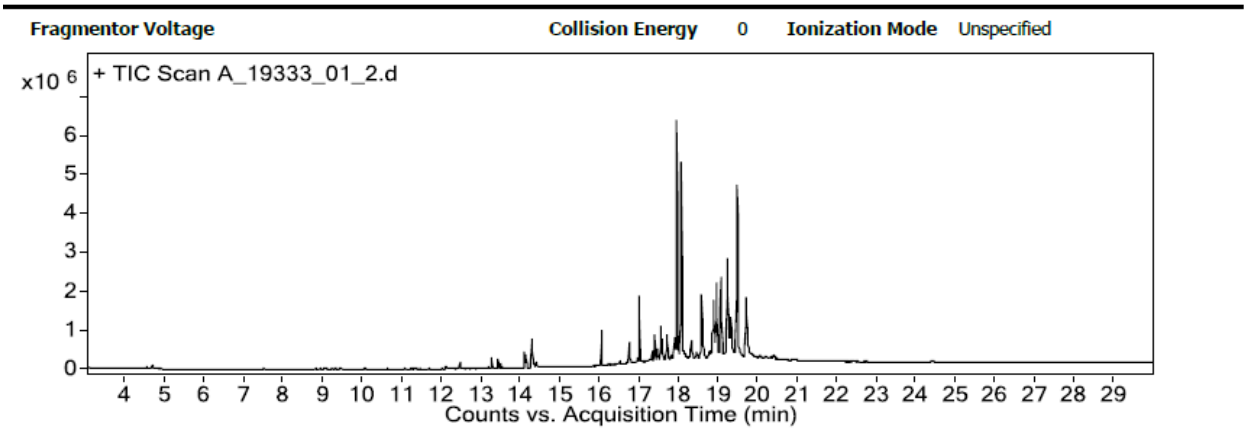
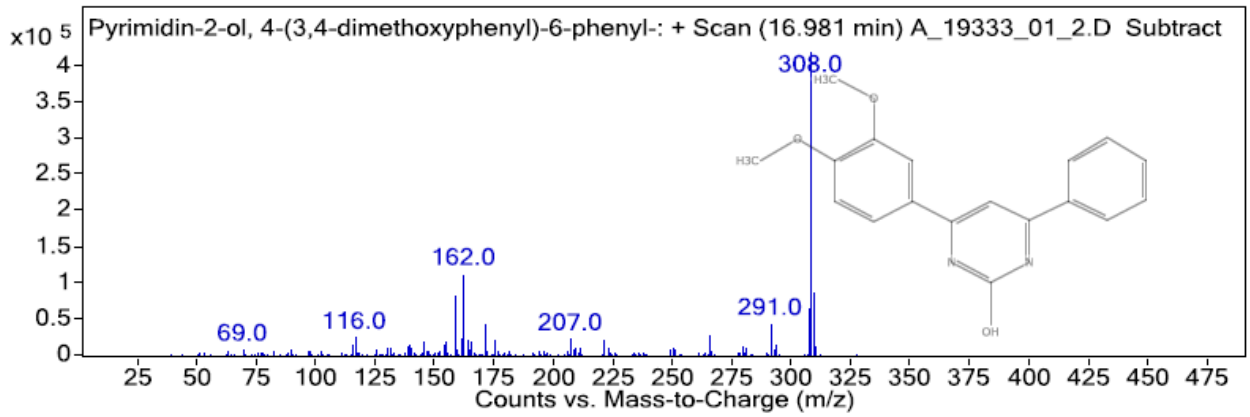
APPENDICIES

Appendix 1: Gas Chromatogram of the Methanol Fraction



Appendix 2: Mass Spectra of Detected Components



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