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A computer aided approach to develop herbal acaricide using *Leucas aspera* and *Cassia alata* against the cattle tick *Rhipicephalus* (*Boophilus*) microplus

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Abstract
Rhipicephalus (*Boophilus*) *microplus* is one of the most important tick species affecting the cattle population. It can cause severe economic loss to cattle and dairy production worldwide and can be responsible for the transmission of many protozoan diseases. Chemical acaricides that are used to control ticks causes resistance development and also leads to deleterious effects on the environment in terms of residues. This served as the driving force to develop herbal acaricides using *in silico* studies. *Leucas aspera* and *Cassia alata* are common plants in India with proven medicinal properties. Docking studies using Discovery accelrys studio was carried out using the active principles present in the two plants on the two target proteins from *R. (B). microplus* namely Boophilin and Triosephosphate isomerase (TIM).The computational studies showed good interaction between the target proteins and active principles of the two plants. The Pharmacokinetic and pharmacodynamics prediction also showed the efficacy of the drug molecules without side effects. The study shows the positive synergistic role of both the plants as potential herbal acaricide. However, the results need to be validated using *in vitro* and *in vivo* studies.

Keywords: Boophilin; *Rhipicephalus* (*Boophilus*) *microplus*; Triosephosphate isomerase (TIM) - drug discovery *–in-silico*

Introduction
Ticks belong to class Arachnida, constituting a major threat to the cattle industry in tropical and subtropical countries. In India, 106 tick species have been reported among which *Rhipicephalus microplus* and *Hyalomma anatolicum* are the most prevalent and hazardous [6]. *R. (B) microplus* mainly infests cattle, deer and buffalo and occasionally infest other hosts like horses, goats, sheep, donkeys, dogs, pigs and some wild mammals.
R. (B) microplus is responsible for transmission of Babesiosis and Anaplasmosis in cattle and also causes severe economic losses due to reduced body weight and milk production, and damage to skin and hides.
Tick control has become a challenge to researchers and hence novel approaches are essential. Synthetic acaricides are used for control of ticks. But these synthetic acaricides possess harmful residual effects on milk and meat which is unsafe for human and animal consumption. The development of resistance to synthetic acaricides is a major problem of tick control.
Ticks are strictly blood feeders. They avoid host haemostatic system through expression of enzyme inhibitors targeting proteolytic reactions of the coagulation and complement cascades. Boophilin found in the midgut of *Rhipicephalus* (*Boophilus*) *microplus* is a 2- Kunitz multifunctional inhibitor which blocks thrombin mainly but also interacts with elastase, kallikrein and Factor (F) FXIa leading to inhibition of platelet aggregation and fibrin formation. Boophilin plays a major role in maintaining the midgut micro environment at low haemostatic and inflammatory tonus. This helps the ticks to digest the blood meal which is crucial for metabolism and egg development. Boophilin is the first tick midgut FXIa anticoagulant that inhibits thrombosis [1].
Triosephosphate isomerase (TIM) is an essential enzyme in glycolytic and gluconeogenesis pathway. It catalyses the interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. It is essential for energy metabolism in ticks. Several studies have analyzed the potential of TIM in drug development against various endoparasites [10].
Boophilin and Triosephosphate isomerase were used as targets in the present study.

Plants are habitually used for animal healthcare from ancient times and promising effect is proven. A number of plants were screened for acaricidal activity by researchers including Neem, *Annona squamosa*, *Calotropis procera*, *Lippia gracilisa* [8]. *Ricinus communis* etc. [4].

Leucas aspera is known for its anti-inflammatory, anti-thrombotic, anti-oxidant, anti- carcinogenic properties [7] whereas *Cassia alata* is known for its antifungal, laxative, purgative and antioxidant activity [11].

Leucas aspera consists of phyto constituents which include phytosterols, oleanolic acid, ursolicacid, saponin, squalene, glycerine, triterpenoid, thymine, catechin, azulone, and caryophyllene. *Cassia alata* holds phytochemicals namely aloe emodin, emodin, rhein, anthraquinone, chrysophanol, tannin, chrysarobin, beta-sitosterol and terpenoid. Among the above mentioned phytochemicals those which follow LIPINSKI's Rule of five were chosen as ligands in the present study [2].

Materials and Methods

In silico analysis

In silico research is very advantageous as it decreases the need for lab work and clinical trials by helping in screening of drug candidates more effectively. Hence in the present study, two plants were selected to explore their combined acaricidal activity against the cattle tick R. (B.) micro plus using Discovery Studio Software as a tool.

Ligand preparation

Various phytochemicals present in *Leucas aspera* and *Cassia alata* (Fig.1a and 1b) were selected from Duke's phytochemistry and IMPPAT database. 3D Structures were retrieved from PubChem database. (Table 1 and 2).

PubChem URL: https://pubchem.ncbi.nlm.nih.gov

IMPPAT URL: https://cb.imsc.res.in/imppat/home

Table 1: List of Phytochemicals (which follows Lipsinki's rule of five) of *Cassia alata*

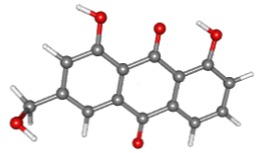
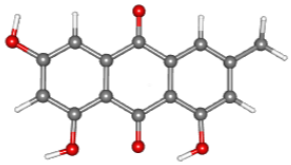
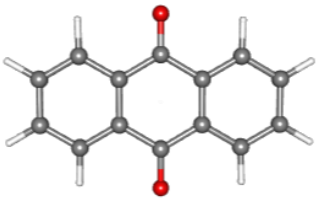
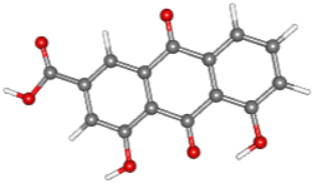
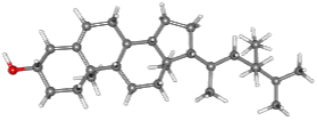
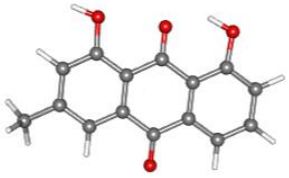
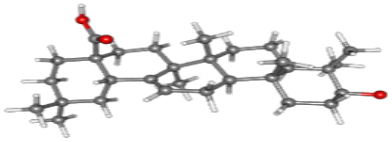
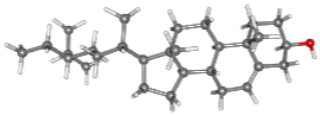
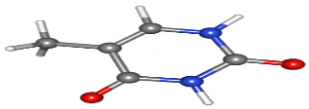
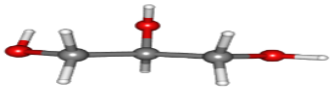
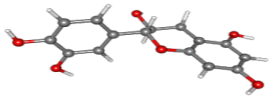
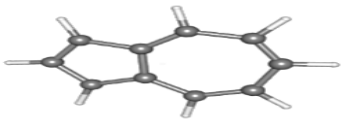
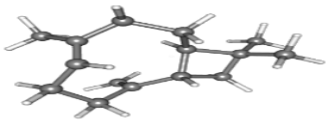
Pubchem ID	Ligands	3D structure
10207	Aloe emodin	
3220	Emodin	
780	Anthraquinone	
10168	Rhein	
222284	Beta sitosterol	
10208	Chrysophanol	

Table 2: List of Phytochemicals (which follows Lipsinki's rule of five) of *Leucas aspera*

Pubchem ID	Ligands	3D structure
10494	Oleanolic acid	
124823816	Phytosterol	
1135	Thymine	
753	Glycerine	
9064	Catechin	
9231	Azulene	
5281515	Caryophyllene	

Target protein

Protein Data Bank is a repository database of three dimensional structural data of proteins and nucleic acids. Target related data was taken from PDB database.
URL:http://www.rcsb.org/pdb

Accelrys discovery studio

Docking study of the target protein molecule and selected bioactive compounds were done using the software Accelrys discovery studio. Discovery Studio software version 4.0 is very useful in the areas of drug discovery and material science.
Docking was done as per the discovery studio protocol.

Docking steps

- The target protein molecule was imported, target protein was prepared and binding cavities were detected.
- Ligand molecule was imported and prepared.
- Docking was done by selecting the ligands against the receptor site.

Prediction of pharmacokinetic properties

The ligands with high dock scores were taken further for pharmacokinetic studies. Pharmacokinetic studies was done using web tool Swiss ADME. The absorption, distribution, metabolism and excretion related information, drug likeness and medicinal chemistry easiness can be gained from Swiss ADME analysis.

A Simplified Molecular Input Line Entry System (SMILES) was collected for the selected ligands from PubChem database. The canonical SMILES were then entered in Swiss ADME web tool for prediction of pharmacokinetic properties.
URL: www.swissadme.ch/

Prediction of pharmacodynamics profile

Pharmacodynamics profile of the ligands with high dock scores was predicted using the PASS online web tool. Prediction of activity spectra of substances is the main function of PASS online web tool. Similar to ADME prediction canonical SMILES obtained from PubChem database was entered in PASS online predictor for analysis. The toxicity prediction was carried out using tox Tree software.
URL: www.pharmaexpert.ru/passonline/

Results

***In silico* analysis (Table 3 and 4)**

Protein: Triosephosphate isomerase (3TH6)

The structure of Triosephosphate isomerase (3TH6) is presented in Figure 2a.

Output

Sixty nine poses were created by Accelerys Discovery Studio and the best docking scores were noted. Results were saved in the output file and it is given in (Table.3). Interaction is shown in Figure 3a and 3b.

Table 3: Interaction with Triosephosphate isomerase

Pubchem ID	Ligand	Lib Doc score	PMF
9064	Catechin	108.291	-57.0167
10207	Aloe emodin	104.65	-76.5048
10168	Rhein	102.472	-132.596
3220	Emodin	98.9344	-46.4222
124823816	Phytosterol	97.5147	-11.0579
10208	Chrysophanol	96.5561	-73.7423
10134	AC1L1UKB	93.5297	-10.6317
222284	Beta sitosterol	87.5651	-60.1211
1135	Thymine	64.4748	-82.5164
10494	Oleanolic acid	61.5851	-30.782

Protein: Boophilin (2ODY)

The structure of Boophilin (2ODY) is presented in Figure 2b.

Output

Thirty five poses were created by Accelerys Discovery Studio and the best docking scores were noted. Results were saved in the output file and it is given inTable.4. Interaction is shown in Figure 4a and 4b.

Table 4: Interaction with Boophilin

Pubchem ID	Ligand	Lib Dock score	PMF
10207	Aloe emodin	87.8516	-17.8182
3220	Emodin	79.8266	-36.1991
1135	Thymine	66.1684	-54.0835
9231	Azulene	61.0423	-18.5629

Pharmacokinetic analysis

ADME studies were carried out for the ligands with highest dock scores *viz*. catechin, aloe emodin and emodin. The results are given in the Figure 5a, 5b, 6a, 6b, 7a and 7b respectively.

Pharmacodynamics profile

The Pharmacodynamics profile of the ligands with highest dock scores *viz*. catechin, aloe emodin and emodin is given in fig.8a.8band 8c respectively.

Fig 1(a): *Leucas aspera*

Fig 1(b): *Cassia alata*

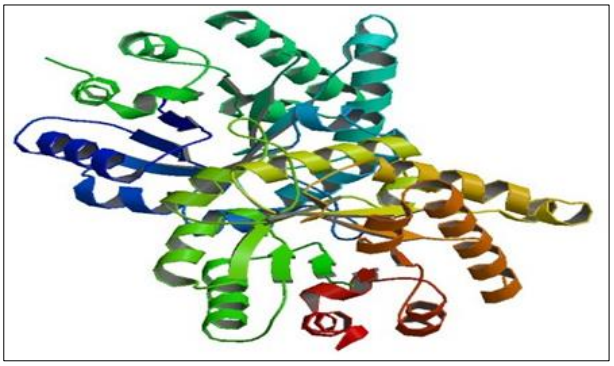


Fig 2(a): Structure of Triosephosphate isomerase (3TH6)

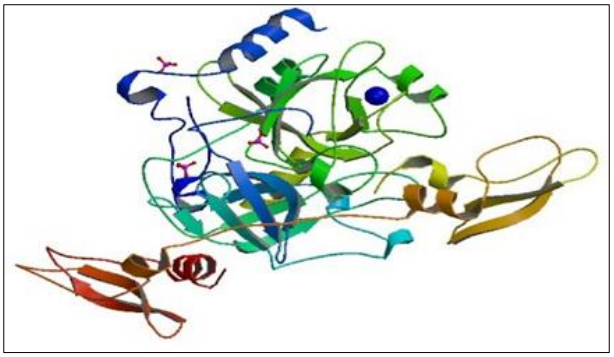


Fig 2(b): Structure of Boophilin (2ODY)

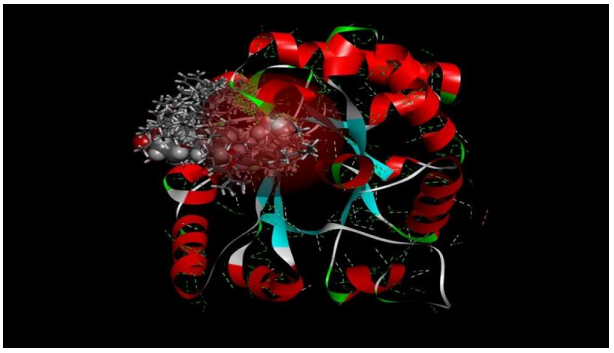


Fig 3(a): 3D Interaction of Triosephosphate isomerase with ligands

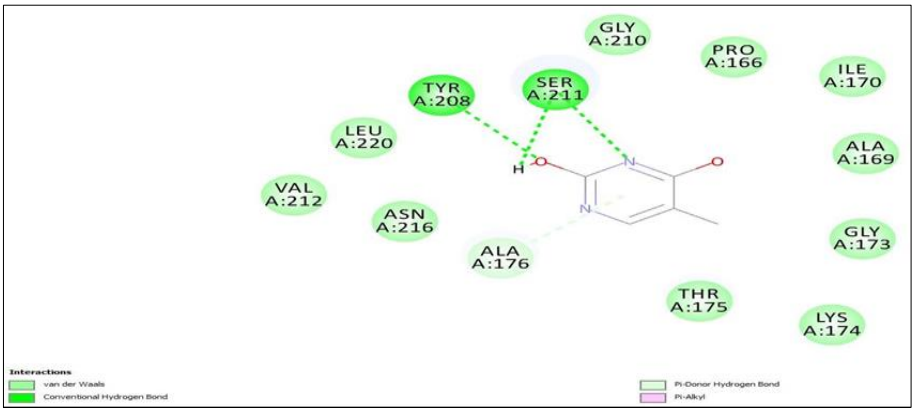


Fig 3(b): 2D Interaction of Triosephosphate isomerase with phytochemicals of *Cassia alata* and *Leucas aspera*

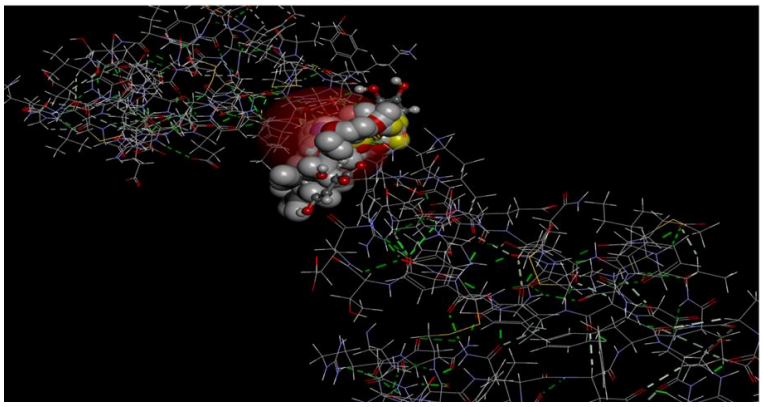


Fig 4(a): 3D Interaction of Boophilin with the ligands

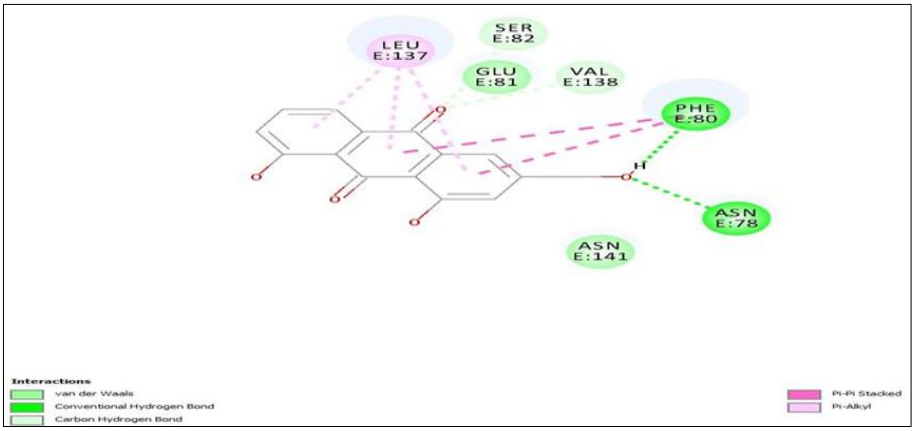


Fig 4(b): 2D interaction of Boophilin with phytochemicals of *Cassia alata* and *Leucas aspera*

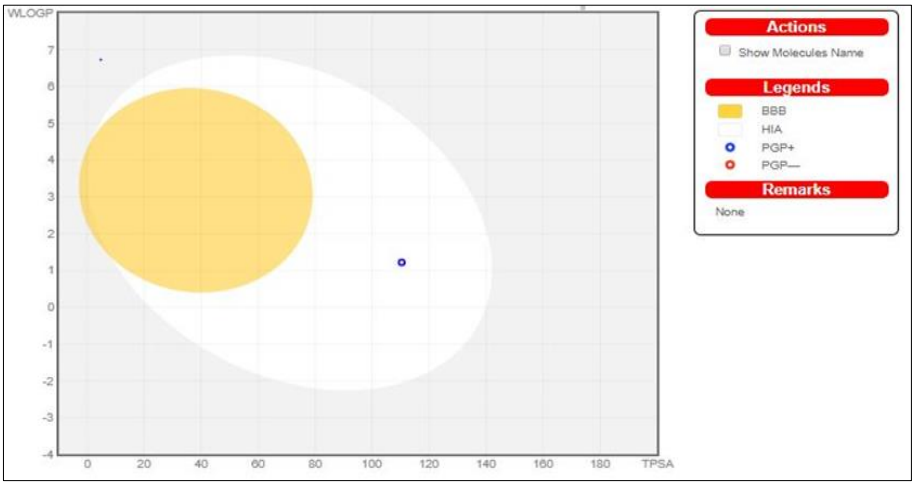


Fig 5(a): Boiled egg predictive model for Intestinal and brain permeation of Catechin

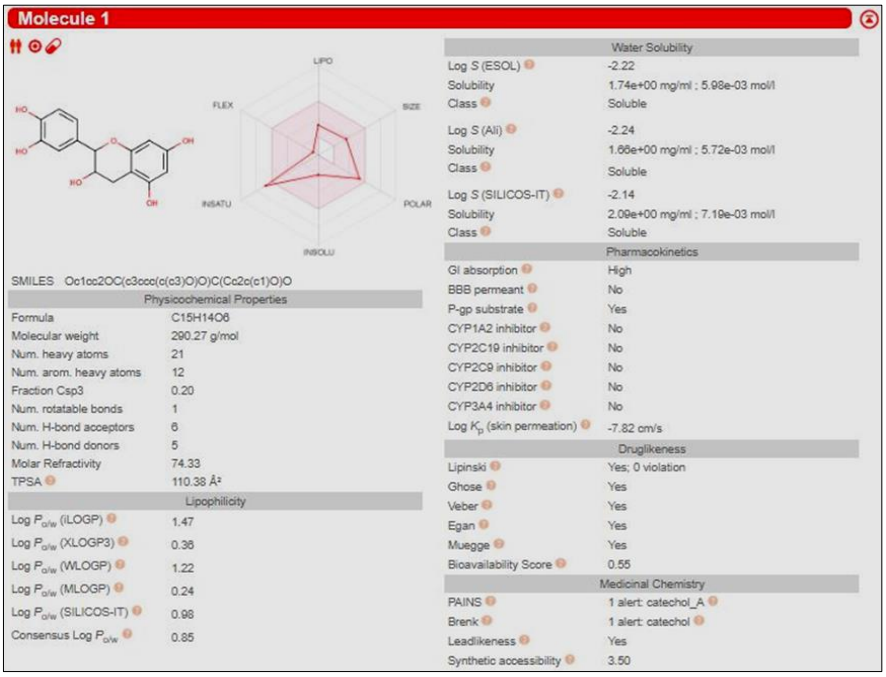


Fig 5(b): ADME profile of Catechin

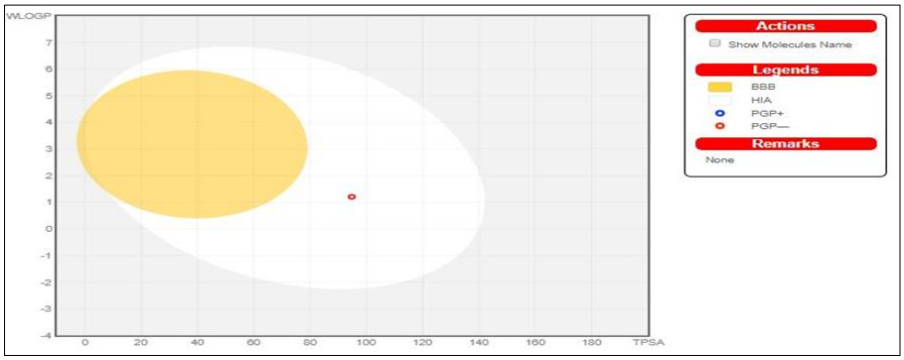


Fig 6(a): Boiled egg predictive model for intestinal and brain permeation of Aloe emodin

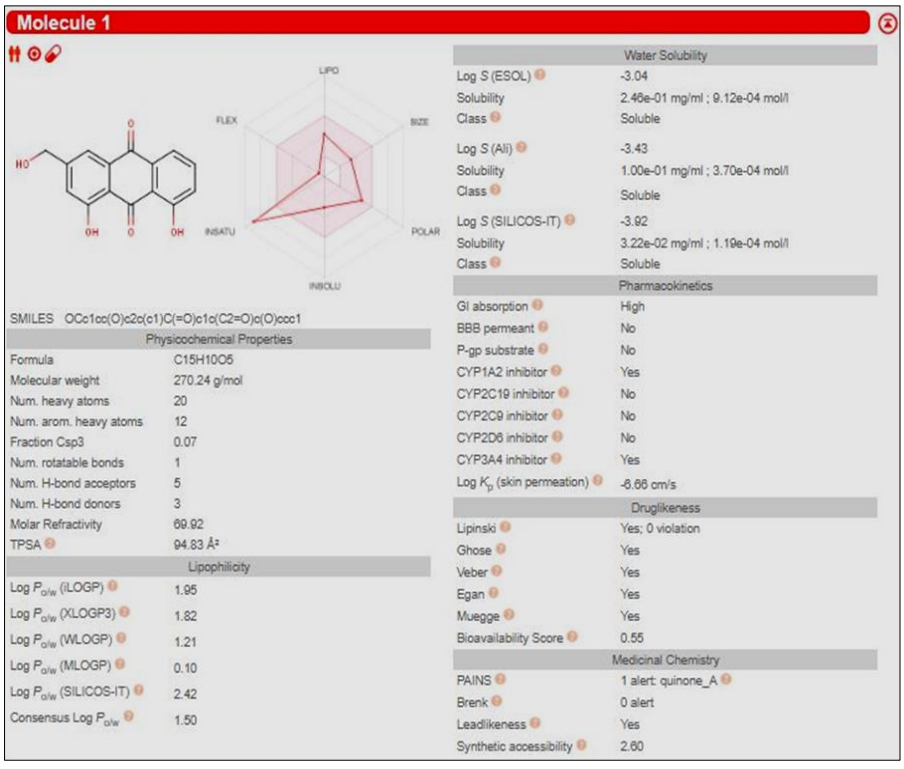


Fig 6(b): ADME profile of Aloe emodin

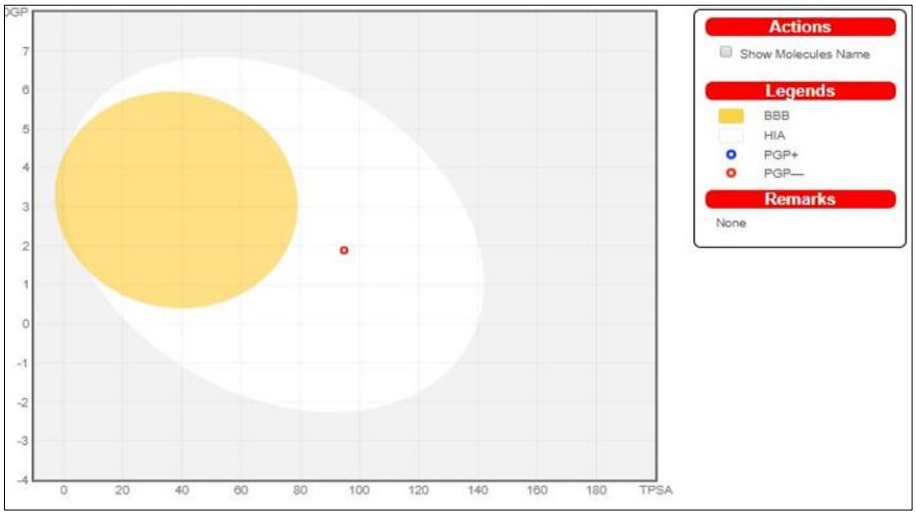


Fig 7(a): Boiled egg predictive model for intestinal and brain permeation of emodin

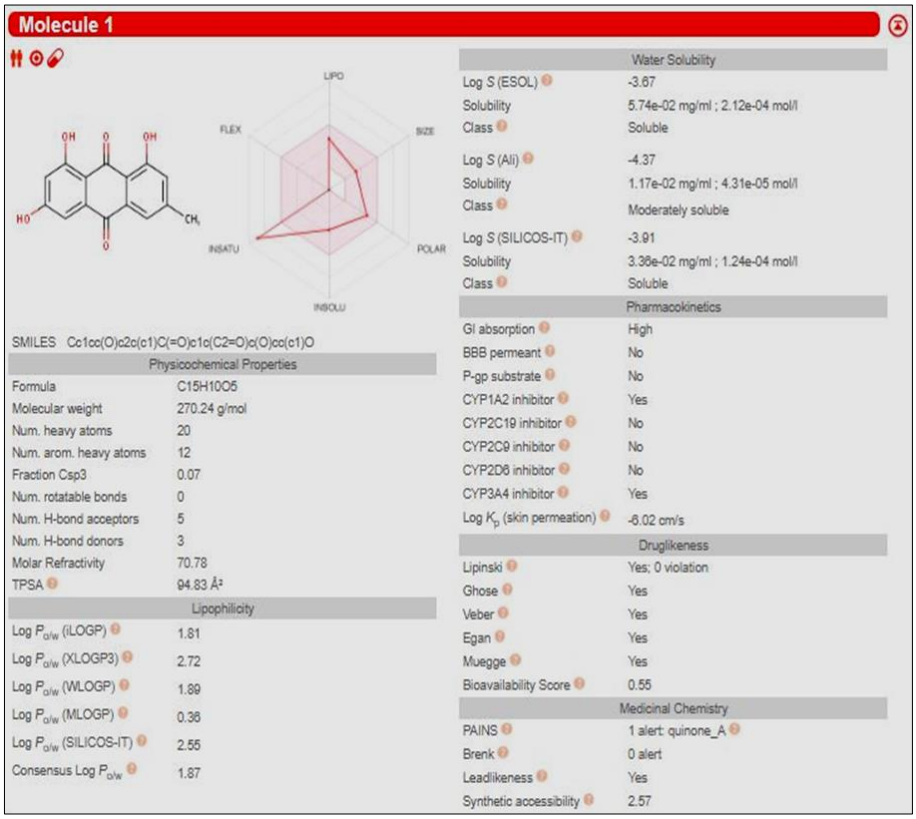


Fig 7(b): ADME profile of emodin

Pa	Pi	Activity
0,983	0,001	Membrane integrity agonist
0,971	0,001	HMOX1 expression enhancer
0,964	0,001	Pectate lyase inhibitor
0,962	0,003	Mucomembranous protector
0,959	0,002	Fibrinolytic
0,959	0,003	TP53 expression enhancer
0,957	0,002	UGT1A6 substrate
0,953	0,001	SULT1A3 substrate
0,951	0,001	Antimutagenic
0,952	0,002	Reductant
0,949	0,002	Sulfotransferase substrate
0,926	0,001	Antihemorrhagic
0,927	0,004	CYP1A1 substrate
0,925	0,003	UGT1A substrate
0,921	0,001	Astringent
0,908	0,003	Lipid peroxidase inhibitor
0,909	0,010	CYP2C12 substrate
0,888	0,005	CYP1A substrate
0,885	0,003	UDP-glucuronosyltransferase substrate
0,884	0,002	UGT2B1 substrate
0,882	0,001	Creatine kinase inhibitor
0,883	0,007	HIF1A expression inhibitor

Fig 8(a): Pharmacodynamic profile of Catechin

Pa	Pi	Activity
0,906	0,011	CYP2C12 substrate
0,881	0,004	NAD(P)+-arginine ADP-ribosyltransferase inhibitor
0,874	0,003	Antimutagenic
0,871	0,004	Antiseptic
0,861	0,002	Tetrahydroxynaphthalene reductase inhibitor
0,851	0,004	Alkane 1-monooxygenase inhibitor
0,850	0,003	Histidine kinase inhibitor
0,861	0,017	Aspulvinone dimethylallyltransferase inhibitor
0,857	0,022	Membrane integrity agonist
0,828	0,002	Laxative
0,830	0,015	Chlordecone reductase inhibitor
0,812	0,004	Phosphatidylserine decarboxylase inhibitor
0,812	0,005	CYP2E substrate
0,807	0,004	NADPH-ferrihemoprotein reductase inhibitor
0,816	0,027	Ubiquinol-cytochrome-c reductase inhibitor
0,791	0,004	Pin1 inhibitor
0,788	0,005	UGT1A9 substrate
0,779	0,007	CYP2B substrate
0,787	0,018	Sugar-phosphatase inhibitor
0,777	0,010	Glucan endo-1,6-beta-glucosidase inhibitor
0,774	0,007	Caspase 3 stimulant
0,777	0,012	Dehydro-L-gulonate decarboxylase inhibitor

Fig 8(b): Pharmacodynamic profile of Aloe emodin

Pa	Pi	Activity
0,932	0,006	CYP2C12 substrate
0,905	0,004	Aldehyde oxidase inhibitor
0,902	0,002	Histidine kinase inhibitor
0,903	0,004	Antiseborrheic
0,899	0,005	Chlordecone reductase inhibitor
0,887	0,003	Antiseptic
0,888	0,014	Membrane integrity agonist
0,875	0,004	UGT1A6 substrate
0,872	0,003	Reductant
0,869	0,003	Alkane 1-monooxygenase inhibitor
0,864	0,003	UGT1A9 substrate
0,860	0,003	Antimutagenic
0,861	0,008	HIF1A expression inhibitor
0,856	0,008	Mucomembranous protector
0,847	0,015	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,834	0,004	Vasoprotector
0,830	0,003	NADPH-ferrihemoprotein reductase inhibitor
0,814	0,003	Tetrahydroxynaphthalene reductase inhibitor
0,823	0,027	Aspulvinone dimethylallyltransferase inhibitor
0,796	0,005	Peroxidase inhibitor

Fig 8(c): Pharmacodynamic profile of Aloe emodin

Discussion

In silico docking of ligands with the targets

The phytochemicals from *Leucas aspera* and *Cassia alata* with two different targets to analyse the acaricidal effect and to explore the possibility of synergism using *Leucas aspera* and *Cassia alata* as herbal acaricides through computer aided approach.

The plants were found to be more effective on Triose phosphate isomerase (3TH6) than Boophilin with a highest dock score of 117.157 and the free potential energy value of -71.4805 indicating potential acaricidal activities of the plant. 174 hydrogen bonds with distance ranging from 1.6 Å to 3.09 Å were formed between the ligands and Boophilin whereas 1046 hydrogen bonds with distance ranging from 0.15 Å to 3.3 Å were formed between the ligands and Triosephosphate isomerase indicating a great interaction.

Pharmacokinetic analysis

The ADME studies carried out using Swiss ADME revealed the pharmacokinetic properties of Catechin, aloe emodin and emodin. The gastrointestinal absorption is high whereas penetration of blood brain barrier is absent in all the three ligands.

Skin permeability varies from -6.02 to -7.82 cm/s suggestive of topical application.

Pharmacodynamics profile

Pharmacodynamics profile of theligands with high dock scores revealed various properties. Catechin bares antimutagenecity, antiseptic, antihemorrhagic, fibrinolytic, anti-oxidant, astringent, membrane integrity agonist, Mucous membrane protecting properties. Aloe emodin and emodin have antiseptic, antimutagenic, membrane integrity agonist, antioxidant properties.

The toxTree results are suggestive of no risk of toxicity as there are no hints of toxicity found in the prediction.

From the *in silico* analysis it is clear that the combination of *Leucas aspera* and *Cassis alata* have shown to possess significant acaricidal properties. The phytochemicals apparently work synergistically and therefore the combined extracts may be desirable drug entity to be considered in developing an herbal acaricide.

However the acaricidal efficacy of *Leucas aspera* and *Cassia alata* against the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* has to be confirmed with *in vitro* and *in vivo* studies.

Conclusion

The dock scores recorded in this study shows good interaction between the active principles of *Leucas aspera* and *Cassia alata* with selected targets of the tick. And the ligands catechin, aloe emodin and emodin hold an excellent drug likeliness, pharmacokinetic properties. Hence this study highlights that the two indigenous plants may have potentials to kill the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*. This finding may be considered as a lead to develop cost effective, safe, eco-friendly herbal acaricide.

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