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Original article

In silico approaches to develop herbal acaricides against *R. (Boophilus) Microplus and In vitro* Anti-Tick activities of selected medicinal plants

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ABSTRACT

In tropical and sub-tropical areas of the world the most damaging pest of the livestock sector are cattle tick, *Rhipicephalus microplus.* The current study was aimed to generate phytochemical derived acaricides to control *Rhipicephalus microplus* populations, to maintain livestock herd production, minimize economic losses and to reduce uses of man-made chemicals acaricides. To achieve this goal, Adult immersion and larval package test were used to determine the feasibility of *Berberium lyceum* and ***Tamarixa aphylla*** against *Rhipicephalus microplus* ticks. Further, an *In silico* technique was employed to discover biologically active substances from both plants using docking method. *Berberium lyceum* and *Tamarixa aphylla* exhibited a reasonably high fatal effect at 40.0 mg/L on egg laying (index of egg laying = 0.19 and 0.19) respectively, thus inhibiting the oviposition (49.5 and 45.1, respectively) and the larval mortality (97% and 93%, respectively). Further, we also used Chem-Draw ultra-software (v. 12.0.2.1076. 2010) to illustrate different structures of38 known bioactive phytochemicals which are discovered in the PubChem database and verify the hypothesis that tick inhibition was linked to acetylcholinesterase (AChE). Barbamunine and rutin from *Berberium lyceum* showed remarkable interaction with RmAChE1 active site residues with docking scores of −9.11 to −8.71 while phytol and dehydrodigallic acid from *Tamarix aphylla* showed comparable docking scores of −7.17 and −7.14 respectively against *Rhipicephalus microplus* acetylcholinesterase protein. Based on obtained result, we believe that *Berberium lyceum* and *Tamarixa aphylla bioactive components* could be potential candidates in the control and management of *Rhipicephalus microplus* and should be studied further as a supplement or replacement for synthetic acaricides.

1. Introduction

Ticks being the blood feeding ecto-parasite causing severe damages by causing tick borne diseases in livestock, wild animals and human (Massard CL et al. 2004; Lopes et al. 2016). Ticks can easily travel along with their hosts resulting in food insecurity and substantial economic losses. The worldwide economic effect of tick and their resulting diseases have been calculated from 13.9 to 18.7 billion USDs (Habeeb, 2010; Karim et al., 2017). The cattle or livestock tick *Rhipicephalus microplus* is a major ectoparasite of cattle that has a broad geographical range and have widespread devastation, especially among sensitive breeds (Pirali-Kheirabadi

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and Teixeira da Silva, 2010). When cattle ticks are not effectively controlled, they can impose permanent losses due to intensive hematophagy and the spread of heamoparasites (bovine babesiosis and anaplasmosis) to herds (Grisi et al. 2014). Anaemia, malnutrition, disablement, loss of weight, reduced milk production (Narladkar, 2018; Rodriguez-Vivas et al., 2018), immunosuppression, systemic abnormalities in their carriers (Reck et al., 2009) and poor leather quality are all symptoms of *R. microplus* infection (Rodrigues and Leite, 2013).

R. microplus is currently controlled by synthetic acaricides such as organophosphates, synthetic pyrethroid and amitraz; however, persistent use of these chemicals has led to the production of new tick variants, sparked widespread concern in the societies and government, thus harms both humans who use these animals' commodities and the animals themselves (Reck et al., 2014).

Researchers are attempting to use plant extracts against ticks to discover new acaricides. Apart from being ecologically safe, they often contain a range of active components with distinct modes of action, these compounds also offer the advantage of delaying the resistance mechanisms (Olivo et al.,2009; Khater et al., 2018; Fayaz et al., 2019). Another strategy to manage ticks is to use secondary metabolites, which can impact cuticle development, diminish growth, maturation, and fertility, or influence behavior without affecting undesired species.

Berberis lyceum Royle is a highly medicinal plant with anti-hyperglycemic, anti-malignant, antioxidant, chemotherapeutic and anti-hyperlipidemic properties and is rich source of beneficial phytoconstituents including berberine and plamitine, which function as strong antioxidants, pro-apoptotic and growth inhibitors against harmful microorganisms and parasites (Jamwal et al., 2016). *Tamarix aphylla* is a member of the *Tamaricaceae* family also possess wide range biological potentials such as antioxidant, antifungal, cytotoxicity, antipyretic, antimicrobial and analgesic properties (Jasiem et al., 2019)., previous research studies have confirmed the availability of ellagic acid in *T. aphylla* and has effect on limiting the growth of insects (Alhourani et al., 2018). Given its potency against other plant arthropod pests' anti-growth activities, it's probable that the interplay of these compounds could make these plants more efficient against cattle ticks.

Acaricides cause immobility and death in arthropods by blocking acetylcholinesterase (AChE), which hydrolyzes acetylcholine (ACh) at cholinergic synapses. The enzymatic activity of AChE, the key enzyme in the neurological system of ticks is also inhibited by the potential activity of carbamate and organophosphate pesticides (Zhou & Xia, 2009; Ribeiro et al., 2012). Arthropod pests have acquired resistance to organophosphate insecticides and a mutation which are naturally occurring has resulted in a modified *acetylcholinesterase* that resistant to organophosphate, thus limiting the potential effects of organophosphate in controlling population ectoparasites (Temeyer et al. 2013). Acetylcholinesterase in *R. microplus* has previously been discovered to be suppressed by phytochemicals from *Cannabis sativa* and *Allium sativum* and *C. serrata* plant extracts (Nasreen et al., 2020; Ribeiro et al., 2012).

The current *in-silico* study takes a computer-aided method with protein model selection, compound screening and small molecule-protein interaction to find best phytochemical candidate in the dataset and propose it as a potential acaricidal drug to control this menace.

The current research study was to determine the anti-tick activity of plant extract on *R. microplus* developmental stages, as well as to increase novel approaches to check tick infection and the spread of tick-borne disease by employing a molecular docking approach to determine special bioactive compounds effective against ticks.

2. Materials and methods

2.1. Tick collection

Adults female *Rhipicephalus microplus* ticks (n = 500) were carefully collected from naturally afflicted animals. The isolated ticks were brought to the Parasitology Laboratory Lab, Abdul Wali Khan University Mardan's and were identified using morphological features (Walker et al. 2003). At proper required temperature and humidity, After the ticks were carefully washed with distilled water thrice they were dried and subsequently sorted into two separate groups. One group (Group 1, n = 420) was used for the adult immersion test (AIT) described below incubated at 10 °C, and the other group (Group 2, n = 50) was held in a desiccator for oviposition assays under standard insectary conditions (i.e., 10% KOH, 28 ± 1 °C and 85 ± 5% RH). These eggs were enabled to hatch and the "larval packet test" was performed on them.

2.1.1. Plant material and extract processing

The above ground parts of the medicinal plant materials were collected from Mardan district of Pakistan's Khyber Pakhtunkhwa (KP) in the month of April and May 2021. Plant material were collected, thoroughly washed and shade dried and finally grounded into fine powders. The plant extracts were prepared using maceration technique, by adding 50 g of powdered plant material in 100 mL of ethanol. In the next step, different dilutions: 40.0, 20.0, 10.0, 5.0 and 2.5 mg/mL were madewhich were further used for tick testing by Adult immersion test (AIT) and larval packet test (LPT).

2.1.2. Adult immersion test

This tests was used to determine different ethanolic dilutions of plant extracts according to the recommendations of Food and Agriculture Organization (FAO) (Sharma et al. 2012). Dose–response assays were carried out using 40, 20, 10, 5 and 2.5 mg/mL concentrations where five ticks per replicate (three replicates of each concentration) were fully immersed in separate solutions for 5 min, i.e., 15 × 5 = 75 adult, fully engorged *R. microplus* females were used.. Distilled water and ethanol was used as a positive control. Before placing in individual plastic specimen vials, ticks were taken from the solution and dried with tissue paper towels (25–50 mm). These tubes were placed at 28.0 °C with 80–85 % humidity in a bio-oxygen demand (BOD) incubator. The laying eggs index and the percent inhibition of productiveness were calculated using formulas Goncalves et al. (2007). The average weight of eggs laid / normal female weight ratio is then calculated using the average weight of females and eggs. After being weighed, the eggs were placed in a glass jars and left them to hatch for 21 days. (IE control group − IE treated group) × 100 / IEcontrol group was used to compute the percentage inhibition of oviposition (% IO). IE stands for index of egg laying while IO means inhibition of oviposition.

2.1.3. Larval packet test

With minor adjustments, the larval package test (LPT) was performed according to FAO standards (FAO, 1984). Each concentration (2.5–40.0 mg/mL) was put to Whatman filter paper rectangles in a proportion of 1 mL. The treated filter sheets were dried on the 37 °C and the rectangles were pleated in the mid and and different sides were fastened with adhesive tapes, making an open-ended packet in which tick larvae could be placed. Approximately 100 larvae were put and sealed into the open end. The packets were placed in a desiccator (Fisher Scientific) at 28 ± 1 °C and 85 ± 5% RH for 24 h. Further the packets were taken out of the desiccator and the mortality was calculated by monitor-

ing live and dead larvae. Triplicates were kept in each acaricide dosage.

2.1.4. Statistical analysis

For the data processing, mixed general linear models were used, which incorporated heteroscedastic variance models to assess the effect of extracts and the repetitions were used as a random effect. Likewise, significant differences were evaluated through the 5% Tukey B test. The lethal concentrations (LC), LC50 and LC90, were assessed using the method of Probit analysis (Finney, 1971) generated by Probit or Logit Analysis. These analyses were processed using SPSS versión 23 (IBM®).

2.2. In-silico screening

2.2.1. Rhipicephalus microplus RmAChE protien Sequence

The *R. microplus* AChE sequence information Accession Id: A0A0F6P2D6 RHIMP were retrieved from uniport (https://www.uniprot.org/) (Wu et al., 2006), a freely available repository protein sequence and process new information (UniProt Knowledgebase tool).

2.2.2. Physical and chemical parameters of RmAChE1 Protein

The *RmAChE1* protein's physical and chemical parameters ProtParam tool was used for calculation. (https://web.expasy.org/protparam/) which investigated the compositions of amino acids, protein subunits surface hydrophobicity (H0), surface charge and thermal denaturation properties. Solubility and thermal gelation characteristics were the only functional properties which was also calculated.

2.2.3. Secondary structure prophecies of RmAChE1 protein

2D structure forecasts of the *RmAChE* protein were predicted using SOPMA server (NPS@: network protein sequence analysis, Cell.Com. (n.d.). Table 3 shows the results of using the PSSpred method by ITASSER (Yang et al., 2015). The method employed frequency distributions of all amino acid in helices and Beta-sheets, which turns included in the resolved by process of X-ray crystallographic protein template to construct secondary structure predictions.

2.2.4. Structures determination through homology Modeling

The protein tertiary structures were determined using different comparative modelling tools: SWISSMODEL (Arnold et al., 2006), PHYRE2 (Kelley et al., 2015), and ITASSER (Roy et al., 2010). PROSA program, Verify3D (https://servicesn.mbi.ucla.edu/Verify3D/), PROCHECK (Laskowski et al., 1996), and ERRAT servers were used to evaluate the query protein's modelled structure for stereochemical quality.. The template used to build the Swiss model of RmAChE1 was a protein from Protein Data Bank with ID:6arxA. The target sequence was selected based on the Qualitative Model Energy Analysis (QMEAN) score (-2.16), Global Model Quality Estimate (GMQE) score of 0.70, percentage of sequence identity of 48.96, and the coverage of 96%. Similarly, the 3D model of the protein RmAChE1 was performed with Phyre2 and ITASSER based on the most suitable template (5X61A, 5X61B) with the value of confidence of 100.0% and coverage of 48% and highest TM-Score of predicted ITASSER model was 0.890. Based on the results of the evaluation, the best model was chosen for further study.

Table 2
Amino acids composition of *ACHE1* protein, estimated by ProtParam tool.

S.NO	Amino acids	*R. microplus*
1	Alanine (A)	7.4%
2	Arginine (R)	5.5%
3	Asparagine (N)	4.5%
4	Aspartic acid (D)	6.4%
5	Cysteine (C)	1.7%
6	Glutamine (Q)	2.7%
7	Glutamic acid (E)	6.7%
8	Glycine (G)	7.6%
9	Histidine (H)	1.5%
10	Isoleucine (I)	3.2%
11	Leucine (L)	8.4%
12	Lysine (K)	2.9%
13	Methionine (M)	1.7%
14	Phenylalanine (F)	4.5%
15	Proline (P)	7.1%
16	Serine (S)	7.6%
17	Threonine (T)	6.2%
18	Trptophan (W)	2.4%
19	Tyrosine (Y)	3.0%
20	Valine (V)	9.1%

2.2.5. Active site prediction

Following the development of the final model, the potential binding sites of acetylcholinesterase protein in cattle ticks were searched through the selection of software (Version 2019.0102) and the CASTponline server. From three atomic (3D) atomic linkages, the Site Finder feature was utilized to calculate possible sites active in the AChE of the *R. microplus*. CASTp (Joe Dundas et al. 2006), an online tool for determining some of a protein's structural qualities such as identifying, queuing and measuring over-concave regions in a 3-dimensional structure of the RmAChE protein. Outstanding residues have been identified based on packaging.

2.2.6. Ligand preparation for docking studies

Researchers used the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) to find different molecules's from the previously reported from *Berberium lyceum* Ahmed et al (2017) and *Tamarixa aphylla* (Jan et al 2019) that can act as acetylcholinesterase constraints. Total 38 compounds were discovered. Chem-Drawultra was used to create different chemicals structures, which were then saved in mol format and open in molecular operating environment

Table 1
RmAChE1 protein physicochemical parameters.

Name of Organism	Molecular Weight (MW)	Sequence length	theoretical Isoelectric point (pI)	Extinction coefficient (EC) (assuming all pairs of Cys residues form cystine)	Extinction coefficient (EC) (assuming all Cys residues are reduced)	estimated half-life	instability index (II)	grand average of hydropathicity (Gravy)	number of negative residue (R)	number of negative residue (+R)	aliphatic index (AI)
R. microplus	65709.82	595	4.76	10,4445	10,382	30	50.47	−0.251	78	50	78.94

Table 3
R. microplus larvae subjected to whole-plant extracts LC_{50} and LC_{90} values.

Extract	LC_{50}	LC_{90}	Slope	$\chi 2$ (df)	R^2
Berberium lyceum	49.14(0–152.82)	188.05 (110.13–1882.64)	0.25	16	0.70
Tamarix aphylla	77.75 (23.53–127.75)	254.09 (186.46–430.54)	0.54	16	0.61

d.f. degrees of freedom, SE standard error, The fatal concentration at which 50% of R.microplus died is known as the LC50. With a 95 percent confidence interval, LC90 is the fatal concentration at which 90% of *R.microplus* died, R2 is the coefficient of determination.

(MOE) (Chemical Computing Group Inc. Canada, 2016). The automatic MOE parameters were utilized to change the molecular docking structures and to perform 3D protonation and power reduction.

2.2.7. Protein preparation and molecular docking:

The protonated *R. microplus* AChE model was then utilized with default settings to perform energy optimization using MOE (Chemical Computing Group Inc. Canada, 2016). MOE program was used to examine protein–ligand interaction and to analyze 2D structures and 3D structures.

3. Results

At dose of 40.0 mg mL^{-1}, the extracts of *B. lyceum* and *T. aphylla* were particularly efficient against the larvae of tick *R. microplus*. *T. aphylla* had an LC_{50} of 77.75 mg/mL and *B. lyceum* had an LC_{50} of 49.14 mg/mL while *T. aphylla* had an LC_{90} of 254.09 mg/mL and *B. lyceum* had an LC_{90} of 188.05 mg/mL confirming the higher efficiency of this extracts (Table 3). As the dosages of the two plant extracts increases, it result decrease in tick number due to mortality increased (Fig. 1b).

When adult females of *R. microplus* were subjected to a 40.0 mg/mL ethanol extract of *B. lyceum* and *T. aphylla*, there was a significant decrease in oviposition ($p < 0.05$) as they were subjected to comparison of different groups by using distilled water and ethanol (Table 4). The Index of egg laying for *B. lyceum* extract was 0.19 ± 0.01 g (mean ± SE) at maximum dose tested (40.0 mg/mL) compared to 0.36 ± 0.01 g for water control. At the same dosage, the Index of egg laying for *T. aphylla* extract was 0.19 ± 0.008 g vs 0.36 ± 0.01 g for controlling of water. When fewer eggs were deposited, inhibition occurred as demonstrated by a lower IE value. At maximum concentration tested (40.0 mg/mL), the *B. lyceum* extract decreased oviposition (% IO) by 49.55 ± 3.57%, compare to 0% for water control. At the same dose, the *T. aphylla* extract inhibited oviposition by 45.1 ± 62.40 percent compared to 0% for water control (Table 3). The percentage of IO increases in direct proportion to the amount of plant extract present (Fig. 1a).

3.1. In-silico screening

3.1.1. Physio-chemical Classification

The *R. microplus* Acetylcholinesterase protein sequence was retrieved using Universal Protien (UniProt) database (Wu et al., 2006). UniProt is a publicly available database of protein sequence and functional information that allows researchers to learn more about the quality, depth and correctness of their particular proteins through a number of cross-references and publicly available

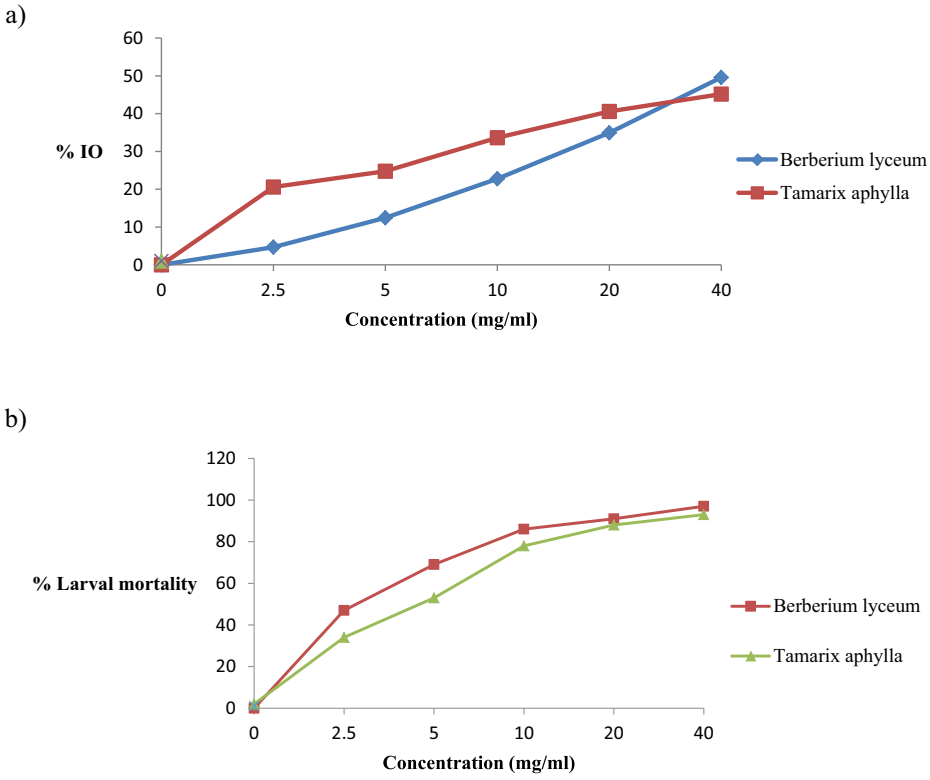


Fig. 1. After treatment with two botanical extracts, (**a**) dosages curve for percentage inhibition of oviposition (% IO) in adult female ticks. (**b**) Dose mortality curve for *R. microplus* larvae after treatment with two plant preparations.

Table 4
Effects of extracts of *B. lyceum* and *T. aphylla* (2.5–40.0 mg/mL) on (mean ± SE) egg laying capability of adult females & larval mortality (%) of cattle tick compared between concentrations at 24 h post exposure.

Extract	Concentrations (mg/mL extract)	Index of egg laying (IE)	inhibition of egg laying (% IEL)	differences between concentrations (%IEL)	% Larval mortality	differences between concentrations (%mortality)
Berberium lyceum	Control H2O	0.36 ± 0.01	5.61 ± 2.61	a	0 ± 0	A
	Control C_2H_5OH	0.35 ± 0.01	3.79 ± 2.04	a	10.00 ± 2.880	A
	0.5	0.36 ± 0.005	4.66 ± 1.99	a	49.00 ± 5.19	B
	5	0.33 ± 0.03	12.43 ± 7.03	a	69.33 ± 8.14	C
	10	0.29 ± 0.01	22.75 ± 4.42	b	85.66 ± 2.08	D
	20	0.25 ± 0.01	34.93 ± 4.55	c	90.66 ± 4.04	de
	40	0.19 ± 0.01	49.55 ± 3.57	d	97.00 ± 3.60	E
Tamarix aphylla	Control H2O	0.36 ± 0.01	−0.77 ± 4.38	a	1.66 ± 2.08	a
	Control C_2H_5OH	0.36 ± 0.02	0.01 ± 0.750	a	5.00 ± 2.510	a
	0.5	0.28 ± 0.02	20.58 ± 6.81	b	33.66 ± 1.52	b
	5	0.27 ± 0.01	24.76 ± 3.35	bc	53.00 ± 5.56	c
	10	0.23 ± 0.01	33.61 ± 3.40	cd	78.00 ± 2.00	d
	20	0.21 ± 0.01	40.57 ± 5.47	de	87.66 ± 5.68	e
	40	0.19 ± 0.008	45.16 ± 2.40	e	93.00 ± 3.60	e

*A difference ($p < 0.05$) within the same parameter is shown by different letters in the separated column.

social media sites (Apweiler et al., 2004). Tables 2 and 3 illustrate the results of utilizing the ExPasy ProtParam tool to analyze the major structure and combine the set of parameters. The *RmAChE1* protein physicochemical parameters include the molecular weight which is 65709.82 Da. Although Expasy's ProtParam tool computed the loss coefficient of *RmAChE* proteins at 280 nm to be 10,4445 in response to the quantity of Cys (Table 1). In solution protein-ligand and protein–protein interactions quantitative analysis occur. The estimated protein concentration and extinction coefficients are useful (Gill and von Hippel, 1989). *AChE1* protein has acidic theoretical isoelectric point (pI). In total, there were about 78 -R and 50 + R charged residues. As an outcomes result, protein has a negative net charge. The *R. microplus AChE1* protein has an instability index of 50.47. If the indicator is<40, the protein is expected to be stable and if it is above 40, it is predicted to be unstable (Idicula-Thomas and Balaji, 2005). Therefore, *AChE1* protein of *R. microplus* was found to be unstable.

3.1.2. Secondary structure predictions of RmAChE1 protein

SOPMA used the default settings for 2D structural characterization. Helixes, sheets and coils make up a protein's secondary structure (Fig. 2). SOPMA reported that *RmAChE1* proteinhave the highest percentage of alpha helices followed by random coils and beta sheets, although beta sheets contributing the least structurally (Fig. 3) (Table 5). PSIPRED server was used to determine the secondary structure (Fig. 4) of *RmAChE1*, obtained from the analysis of PSI-BLAST (Position Specific Iterated - BLAST) output by two feed-forward neural networks (McGuffin et al., 2000). Using sequence data from the PSSpred approach (Yang et al., 2015), which combines PSI-BLAST (Altschul et al., 1997) profile information with seven neural network classifiers from various criteria, ITASSER was also utilized to predict the RmAChE1 secondary protein's structure.

3.1.3. Modeling of the RmAChE1 protein and energy Optimization

The Rm*AChE1* receptor amino acid sequence (Uniprot KB Accession Id: A0A0F6P2D6 RHIMP) was retrieved protein sequence databank because the 3 dimensional structure of the Rm*AChE1* receptor was not available in the protein data bank (PDB). The Rm*AChE1* receptor sequence was subjected to BLAST search against in PDB (Bernstein et al., 1977) to find a protein with a structure that was mostly similar to the query proteins. The protein sequences with maximum sequence resemblance to the query proteins were chosen as templates for further research. Three tools, including Swiss-Model server (Arnold et al., 2006), Phyre2 (Kelley et al., 2015) and ITASSER, were used to create a 3 dimensional model of the *R. microplus* protein *AChE1* utilizing homology structure modelling to determine *in silico* functional insights based on experimentally predicted homologs (Fig. 5) (Ali et al., 2015). In 3D models, energy reduction was employed to eliminate steric interactions

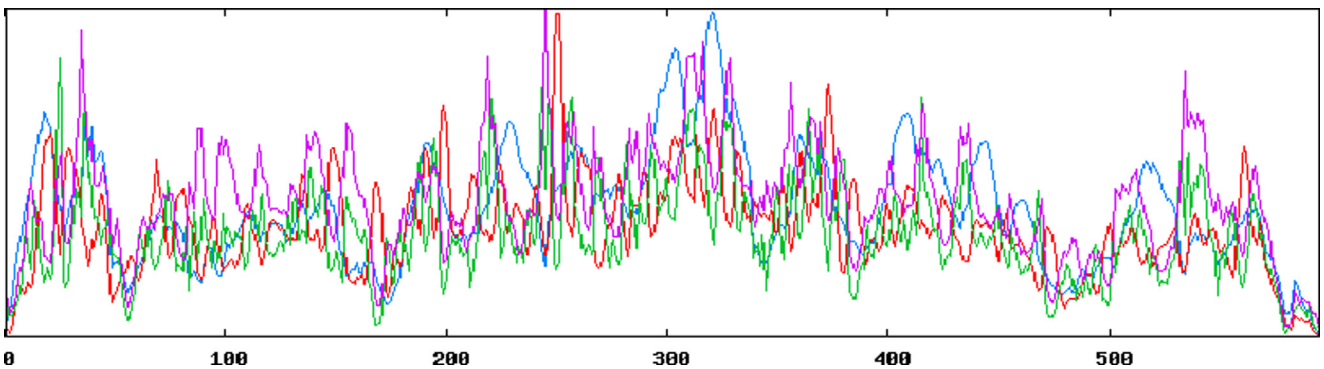


Fig 2. Through the SOPMA server, alpha helices, Beta sheets, turns, and random coils are graphically represented in blue, red, green, and orange colours.

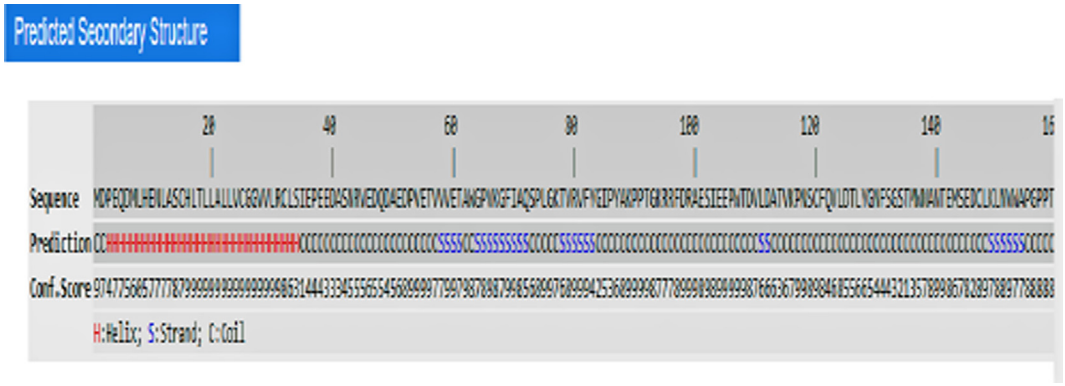


Fig 3. 2D structure of the Rm*ACHE1* protein predicted by I-Tasser tool.

Table 5
SOPMA's Secondary Structure Elements of *RmAChE1*.

Secondary Structure Elements	Standards (%)
ALPHA HELIX (Hh)	47.18
310HELIX (Gg)	0.00
Pi HELIX (Ii)	0.00
BETABRIDGE (Bb)	0.00
EXTENDED STRAND (Ee)	14.92
BETATURN (Tt)	5.24
BEND REGION (Ss)	0.00
RANDOM COIL (Cc)	32.66
AMBIGUOUS STATES	0.00
OTHER STATES	0.0

and strains without compromising the whole structure. To compute and reduce energy, the GROMOS96 force field (Scott et al., 1999) and Swiss-PDB observer were used.

3.1.4. Validation and analysis of the predicted structures

After optimization procedure, the stereochemical clarity of the predict model and precision of the different models of protein were assessed using Ramachandran Map calculations performed with the PROCHECK tool (Table 6). Ramachandran Map's psi and pi distributions, as well as the protein amino acids in the preferred region, indicating that the Rm*AChE1*receptor's projected model is well-built and more dependable as shown in Table 6. Another structure evaluation server, Prosa-web, validated the modelled structures of the *R. microplus AChE1* protein (Wiederstein and Sippl, 2007). The Prosa-web identified normal bond angles in the-modelled 3 dimensional structures. The degree of activeness' of the projected structures was calculated using the Z-score. Swiss-Model, Phyre2, ITASSER, and Z-scores for the modelled 3D structure were −9.44, −9.78, and −7.6, respectively. In current study, all three servers, namely ITASSER, Phyre2, and Swiss-Model, produce comparable results that validate the 3D architectures (Fig. 6). Verify 3 dimensional (3D) model quality assessment (Sahu and Shukla, 2014). Verify 3D to verify the model's quality by demonstrating the conformity of an atomic model (3D) with an amino acid sequence and their score profile (Sahu and Shukla, 2014). With a score of 96.26 %, Phyre2 with 91.43 %, and I-TASSER with 89.08 %, the 3D models completed this evaluation experiment, whereas the pass mark is 80%. Fig. 6 shows the 3D structures that ERRAT has validated. When the data from the Phyre2, Swiss Model and ITASSER models were compared, the Swiss Model was determined to be preferable to the Phyre2 and ITASSER models, and it was selected for future investigation.

3.1.5. Prediction of active sites

For docking research, the projected model binding sites must be identified. Due to the unavailability of the protein structure in the database various receptor binding regions must be anticipated and the interaction area with the largest pocket size is chosen to dock compounds. Using the MOE software's, sitefinding tool was used to calculate the potential binding sites in *RmAChE1* using 3D atomic dimensions of the receptor, 8 best potential binding sites were found. The active site of the target protein was comprised of amino acids GLN122, VAL123, LEU124, ASP125, THR126, LEU127, SER134, TRP137, ASN138, ALA139,TYR173, GLY174, GLY175, GLY176, TYR178, Ser179, GLY180, THR181, LEU184, TYR187, GLU255, SER256, TRP289, THR335, ASN336, SER337, GLY338, GLY339, VAL340, VAL341, ASP342, PHE343, PRO344, TRP384, and PHE385.. The constraint ranges for displaying partial molecular surfaces were found as well as probable active locations for lead binding and docking (Fig. 7) (Goodford., 1985). Potential *RmAChE* receptor sites from *R. microplus* are also searched using CASTp server (https://sts.bioe.uic.edu/castp), which identified some of the geometric properties of proteins such as identifying, distinguishing and assessing concave surface regions on the *RmAChE1* protein's 3D structure (Fig. 8).

3.1.6. Ligand interaction calculation & docking Studies:

The 38 phytochemicals were examined against *R. microplus AChE1* protein target. The *AChE1* docking investigation was carried out using MOE simulate module. The results of the docking study were used to test different variety of docking combinations in order to determine the most accurate binding modes. To identify the inhibitor, a library of phytochemicals from *Berberium lyceum* and *Tamarixa aphylla* is docked with in MOE with distinct active sites of acetylcholinesterase.

To figure out where the ligand was binding, researchers looked at the molecular interactions between protein and each phytochemical. The findings were reviewed after MOE was used to dock a library of phytochemicals from both plants were used as ligands. The high potent inhibitor was determined to be berbamunine from *Berberium lyceum* with docking score of − 9.1067057 (Fig. 9) and successfully binds into the active binding pocket of the targeted protein (Table 7). Other ligands, such as rutin, oxyacanthine and dehydrodigallic acid (Figs. 10–12), all had a high docking score, indicating that they were definitive binding modalities with lower docking energy. Table 7 summarizes the phytochemical docking scores against each protein. The majority of phytochemicals had dock scores higher than −5.5 kcal/mol, according to the Table 7 of dock scores of 38 phytochemicals against target proteins.

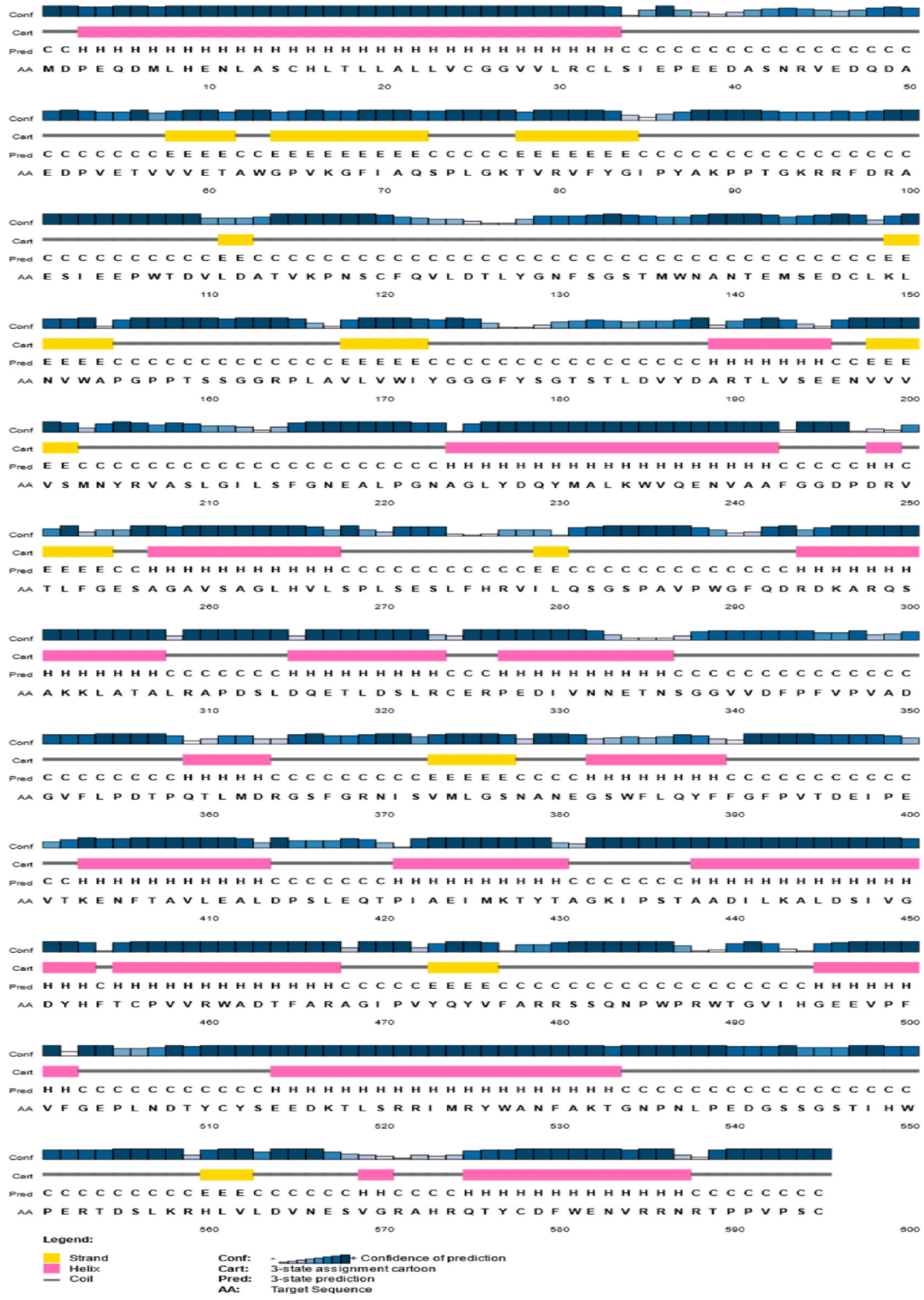


Fig 4. Secondary structure of the *RmAChE1* protein predicted by the Psipred server.

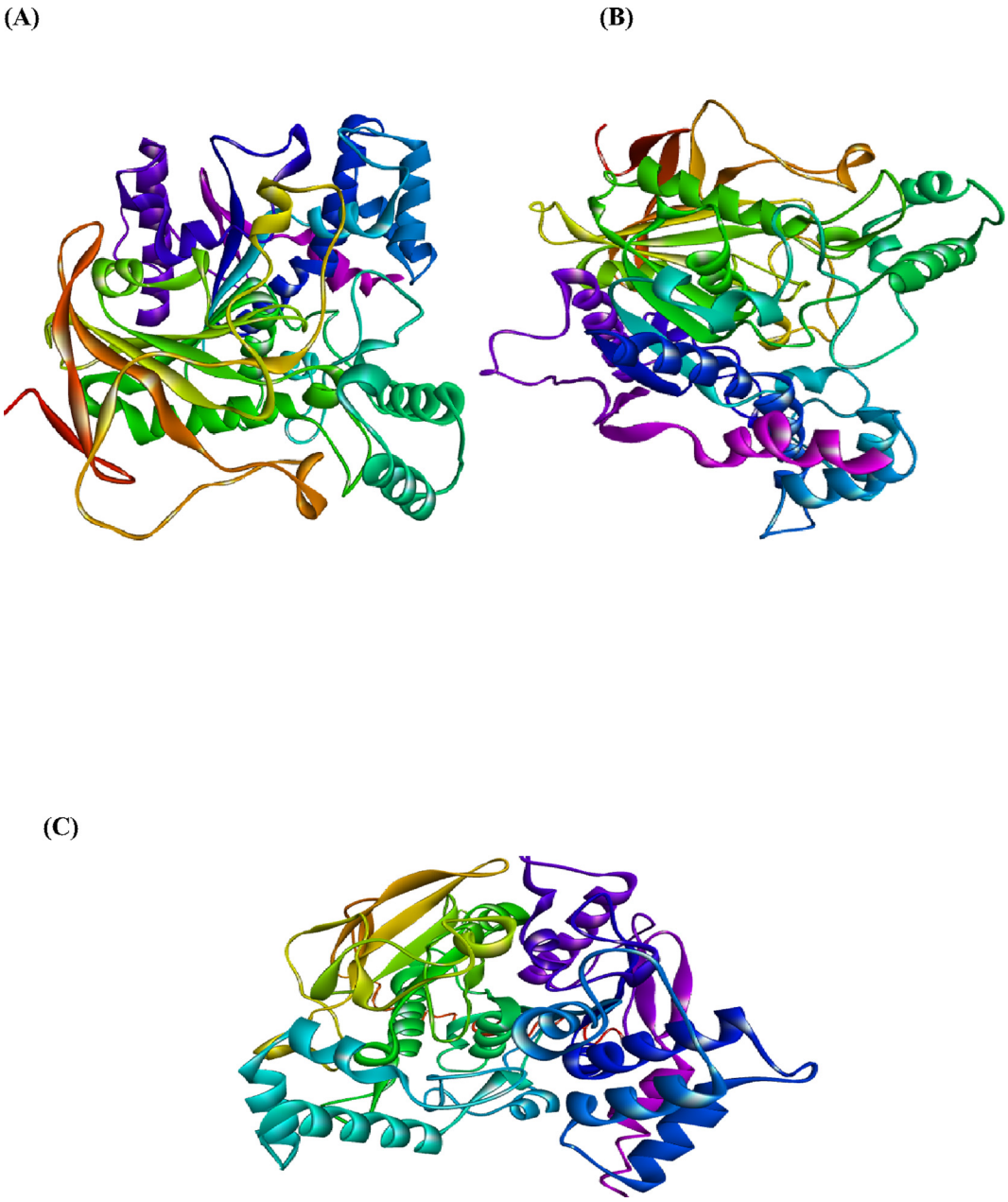


Fig 5. Structure of *RmAChE1* predicted by (A) Swiss Model, (B) Phyre2 and (C) Itasser server Visualized with Discovery Studio.

Table 6
Ramachandran plot calculation using Procheckserver.

Ramachandran plot calculation	Swissmodel server calculation of	Phyre2 server calculation of	ITASSER server calculation of
Number of residues in favoured region	87.0%	84.9%	72.7%
Number of residues in allowed region	11.7%	12.7%	20.0%
Number of residues in outlier region	0.4%	1.3%	2.4%

4. Discussion

This study examined the anti-tick efficacy of *Berberium lyceum* and *Tamarixa aphylla* plant extracts to investigate if new herbal acaricides and bioactive constituents might be developed to combat tick infestation on livestock. Ticks were treated with different concentrations of ethanol extracts (2.5, 5.0, 10.0, 20.0, and 40.0 mg ml^{-1}). *Tamarixa aphylla* herbal extracts had the highest death rate at 40.0 mg ml-1 concentration (93% to 97%). Many herbs have been studied previously for possessing of acaricide properties against mites (Kim et al., 2004; Magi et al., 2006), Culicoides (Narladkar et al., 2006), and ticks (Shyma et al.,2014; Kumar et al., 2016). Due to the successful mass produce of essential oils, the repulsive and anti-parasitic characteristics of several plants have been profitably used beside a variety of parasites (Jaenson et al., 2006).

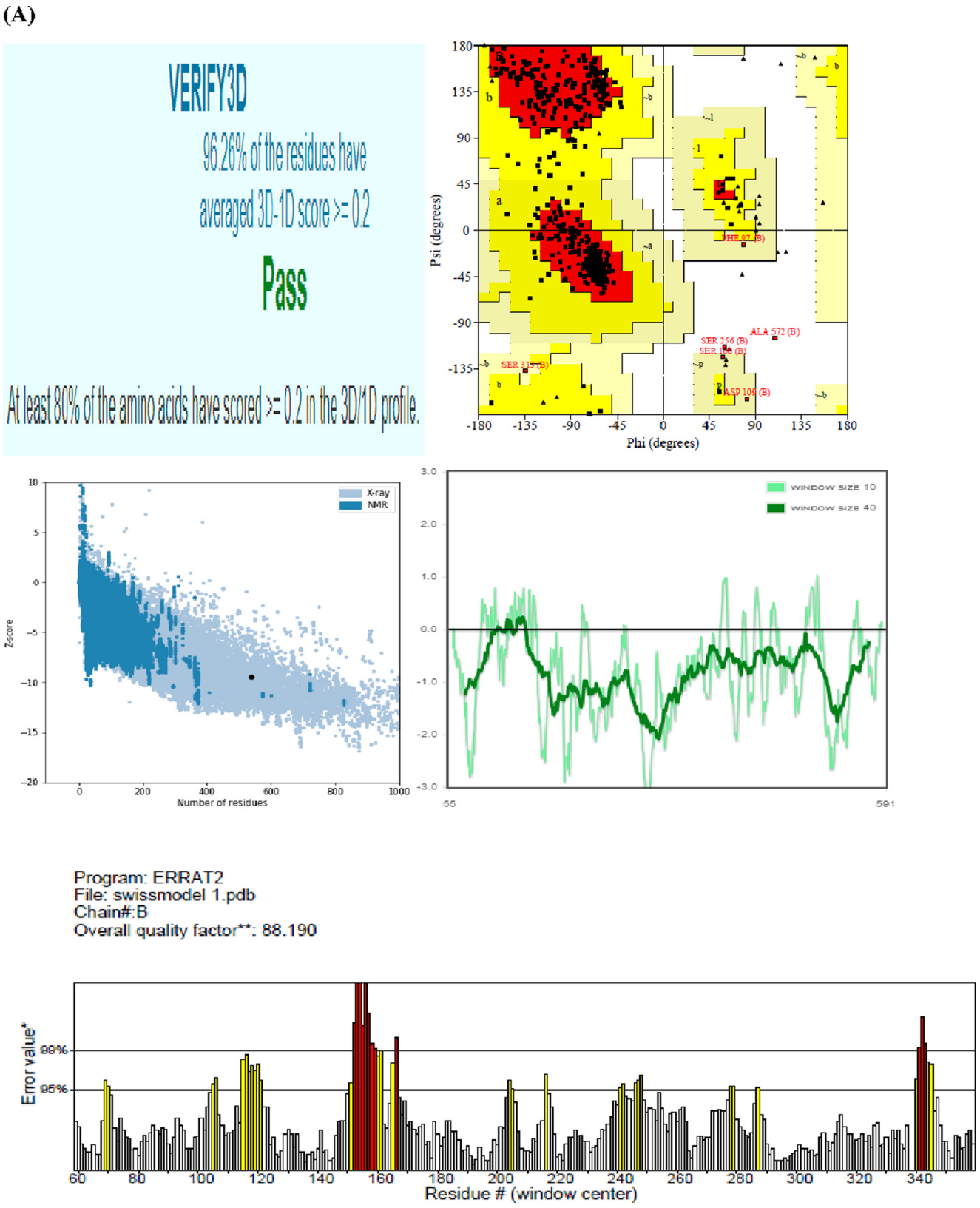


Fig 6. For the confirmation of the *RmAChE1* protein, Ramachandran plots, verify 3D, PROSAweb (Z-socre), and Errat server for (A) Swiss Model, (B) Phyre2 Model, and (C) Itasser Model were used.

(B)

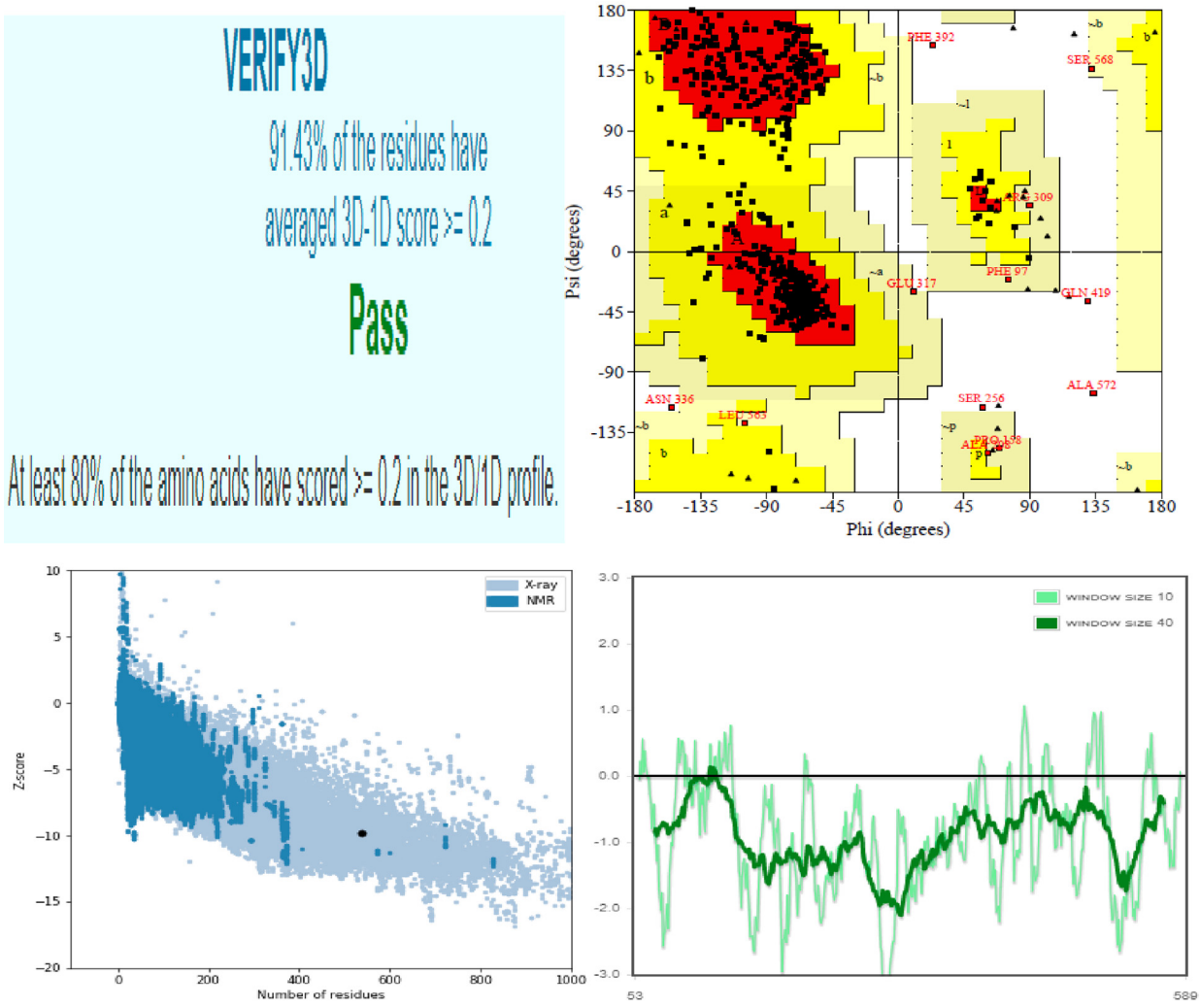
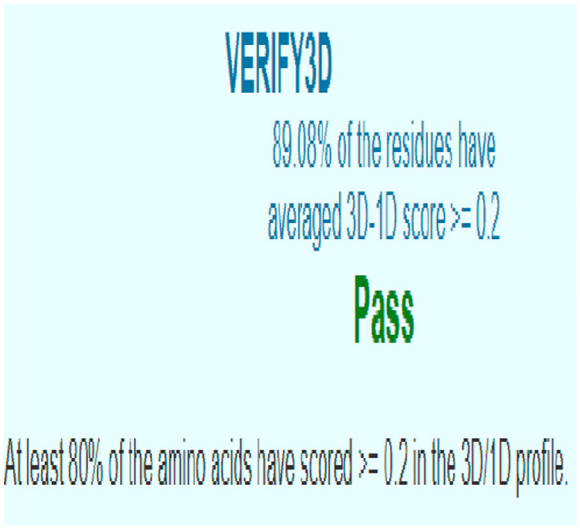
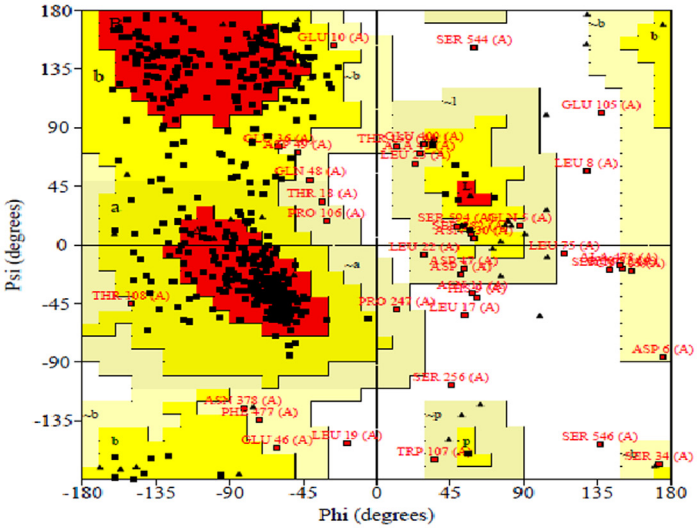
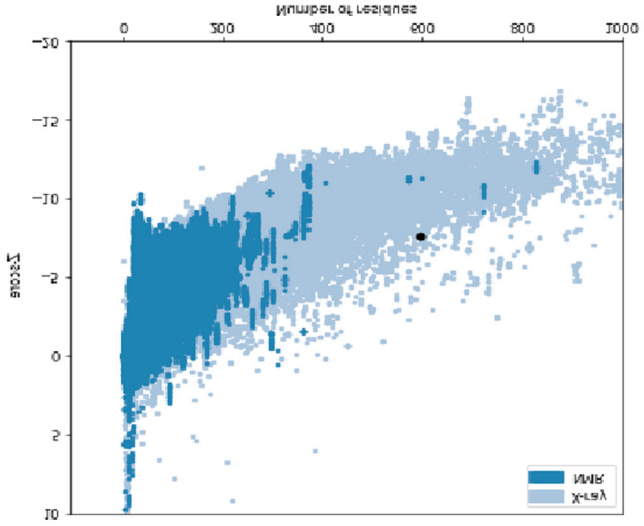


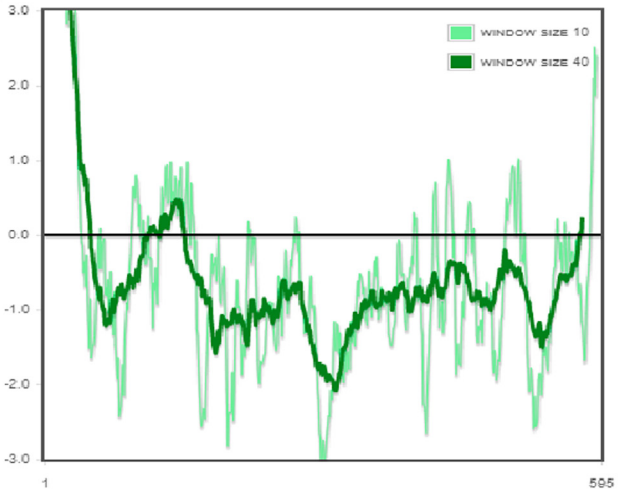
Fig. 6 (*continued*)

(C)









Program: ERRAT2
File: Itasser model.pdb
Chain#:A
Overall quality factor**: 81.431

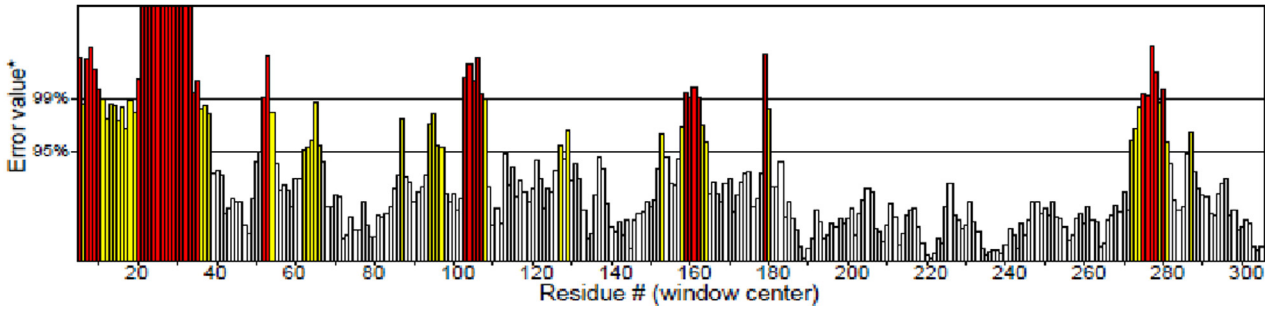


Fig. 6 (*continued*)

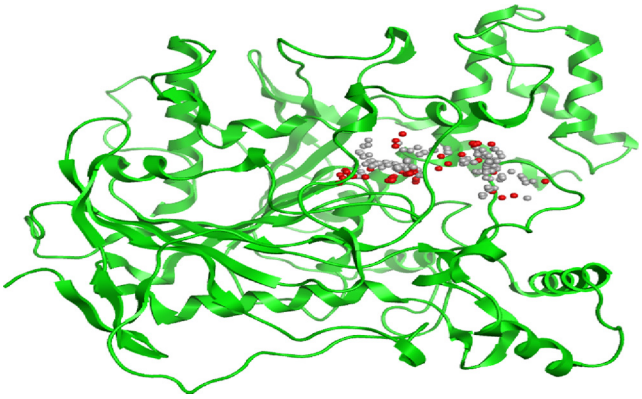


Fig 7. The region with little red and grey balls in the 3D protein structure of *RmAChE* represents the active site.

B. lyceum has been studied for its phytochemical components and biological activity due to its ethno-medicinal usage. Gulfraz et al. (2008) and Ahmed et al., (2009), investigated the phytochemical components and biochemistry as well as bioactivities of its extracts. Alkaloids are the active ingredients in *B. lyceum* in which its roots contain 2.45 % alkaloid (Srivastava et al., 2010; Khan et al., 2010). According to Ikram et al., (1966), "Berbericine, berbenine and berbericinine iodide are three alkaloids found in *B. lyceum*." Miana (1973); Leet et al. (1983); Khan et al. (2010) "isolated and identified different alkaloids from the roots, such as berberine-CHCl3, palmatine-CHCl3, oxyberberine, karakoramine, berbamunine, chenabine, jhelumine, berberine and palmatine". The plant and its phytocompounds have various properties such as antihyperglycemic, anticancer, antihyperlipidemic, antitumor, wound healing and other biological actions are anticoccidial, antimicrobial, antioxidant, immunity strengthening, hypotensive, antiviral, antiurolithic, hepatoprotective and antihelmintic.

Phytocompounds of *Tamarix* plants are used to cure of skin, spleen and sight disorders, toothaches, digestive ailments, abdominal discomfort, animal bites and poisoning (Alzweiri et al., 2011). *T. aphylla* is a hopeful natural source for beneficial and pharmacological discoveries due to its phytoconstituent diversity and high potential. The aqueous ethanolic extract of *T. aphylla* leaves galls was discovered to be an anti-inflammatory and antipyretic mediator (Ali et al., 2019). *Tamaricaceae* has more than 60 chemical compounds that have antibacterial properties against a variety of pathogenic bacteria. For example, *Tamarix boveana* showed antimicrobial activity against six type of bacteria as well as four fungi species (Saidana et al., 2008).

According to Souliman et al., 1991; Orabi et al., 2015, plant extracts of *T. aphylla* contained flavonoids, phenolics, hydrolysable tannins and alkaloids and its leaves have been use to treat different diseases (Marwat et al., 2009; Mahfoudhi et al., 2016). The different phytocompounds found in *T. aphylla* have been identified through several research studies. An one-off isoferulic acid derivative, *aphyllin*, was discovered to have a unique radical scavenging activity and to help human keratinocytes survive (Nawwar et al., 2009). In Saudi Arabia, the alcohol is extract from *T. aphylla* leaves was discovered to exhibit antioxidant, anti-inflammatory, and wound-healing effects.

The space will be filled however, by providing a whole phytochemical window of both plants and thoroughly examining their broad range of biological activity. Our study tried to close that gap. The purpose of this study is to do advance basic scientific and practical research on the pharmacological, biological and acaricidal properties of this plant and of their pure ingredients, particularly alkaloids like berbamunine, (a potent RmAChE1 inhibitor in this study).

Screening chemical libraries in vitro with a confirmed target is a crucial step in finding new drugs. *In silico* screening has aided in diseases control for a number of pathogens including viruses, parasites, bacteria, and fungi (Deng et al., 2014). *In silico* experiments was performed on *R. microplus* AChE1 to better understand the connections of these phytochemicals (found in *Berberium lyceum and Tamarix aphylla*) with *R. microplus* AChE1. Tick survival is dependent on RmAChE1 as shown by its high affinity for target (acetylcholine; ACh) and higher conversion rates than *Rhipicephalus microplus* acetylcholinesterase 2 and *Rhipicephalus microplus* acetylcholinesterase 3 (Temeyer et al., 2010). The development of new acaricides and our understanding of their mechanisms of action are hampered by lack of understanding of RmAChE's crystalline structure (Ferreira et al., 2018). Comparative

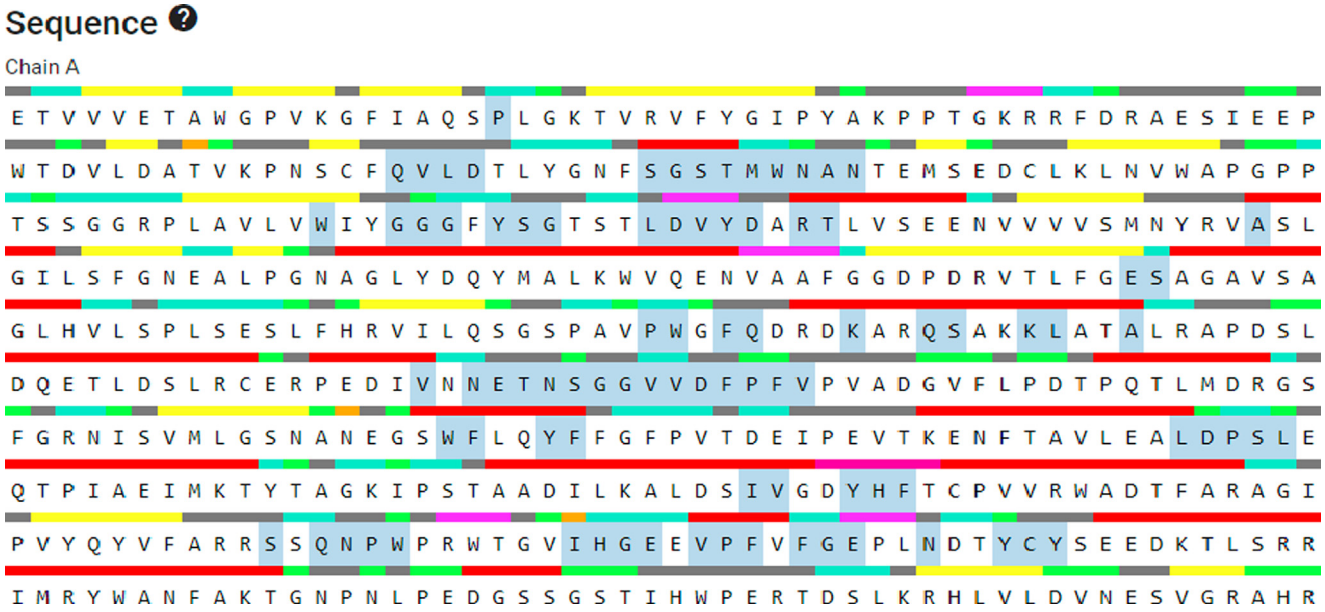


Fig 8. The CASTp server predicted the active site with largest pocket size for binding residues.

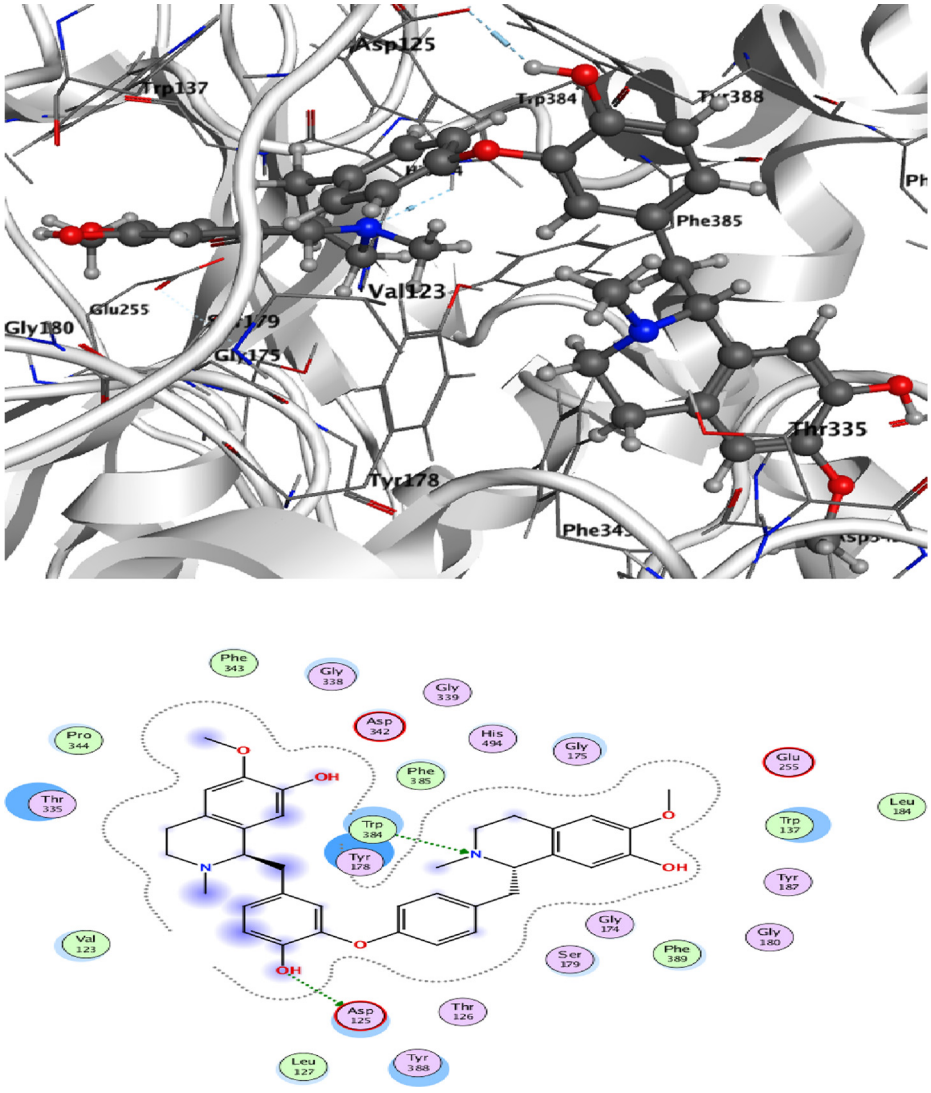


Fig 9. Figure showing Barbamunine's 3D and 2D bonding with *R. microplus*'s *AChE1*.

modelling allows docking technique to be used to analyze antiACh medication molecular interactions at the atomic level, it is critical in overcoming these restrictions (Schmidt et al., 2014).

The docking analysis of *AChE1* was performed in current study using MOE Simulate module (Molecular Operating Environment) program (Version 2019.0102) and docking parameters were set to be as stringent as possible, Placement: Rescoring function 1: London dG, Triangular Matcher. A total of 38 structures of phytochemicals were drawn using ChemDraw software and were used as ligands in the docking study. Berbamunine, from *Berberium lyceum* was found to be the most potential inhibitor with a docking score of "- 9.1067057" (Table 7). The research goes on to look at the compounds with the highest dock scores that show interactions with critical residues. Rutin, oxyacanthine, dehydrodigallic acid, phytol and kaempferol-7,4 dimethylether (Table 7) were also found to be high-scoring phytochemicals from both plants with differing bond interactions.

Due to these acaricidal and repulsive actions on ticks, plant derived bioactive phytochemicals are clearly interesting agents for integrated pest management (IPM) programs. Due to their biosafety and ecofriendly nature, reduced hazards to both flora and fauna the plant derived acaricides has significantly attracted the attention of scientific community. A number of commercial products based on phytocompounds have been produced due to scientific developments. To conclude, information of acaricidal plants widespread in the regional and local environment is now required for establishing tick control and management program that is acceptable, efficient, affordable, approachable, environmentally friendly and community-driven.

5. Conclusion

The anti-tick properties of plant acaricides were assess in the first stage of the study. Different bioassays were used to examine the acaricidal abilities of "*B. lyceum and T. aphylla*," two highly medicinal plants in Pakistan. The killing of *R. microplus* larvae has an influence on the reproductive capacity of adult females. Our research finding give away for further research study into whether those plants could be utilized to make natural medicine. This method is essential for dealing with cattle infestations and populations of *R. microplus* which are resistant to synthetic acaricides.

The second part's goal was to see if understanding the three-dimensional structure of small molecules may help researchers to identify, design and produce acaricides using computer-aided methodologies. In this study, molecular docking approach was used to find possible acetylcholinesterase inhibitors. Using homol-

Table 7
Table displaying the docking scores for various *B. lyceum*and *Tamarix aphylla* compounds.

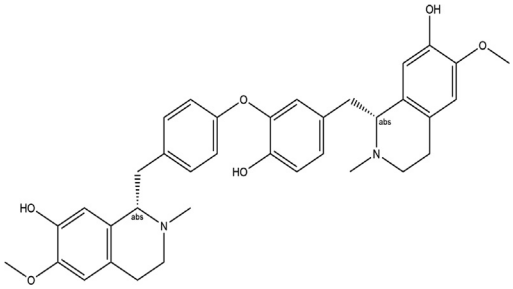
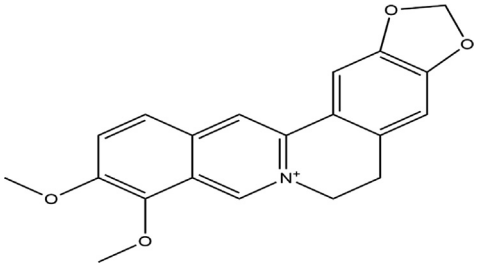
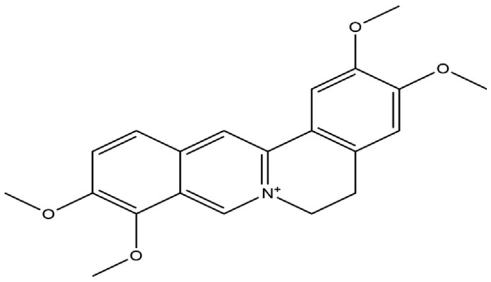
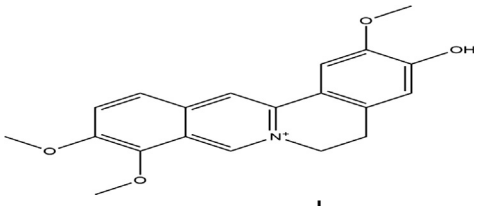
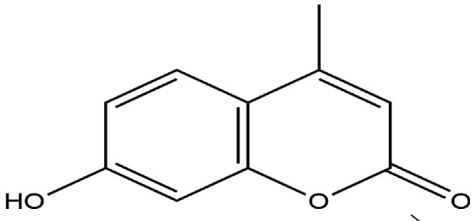
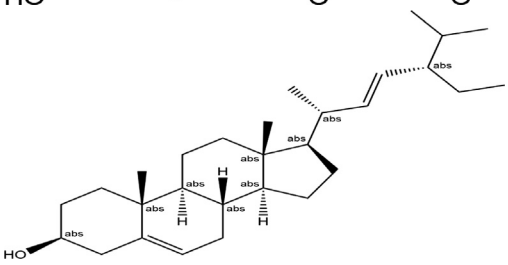
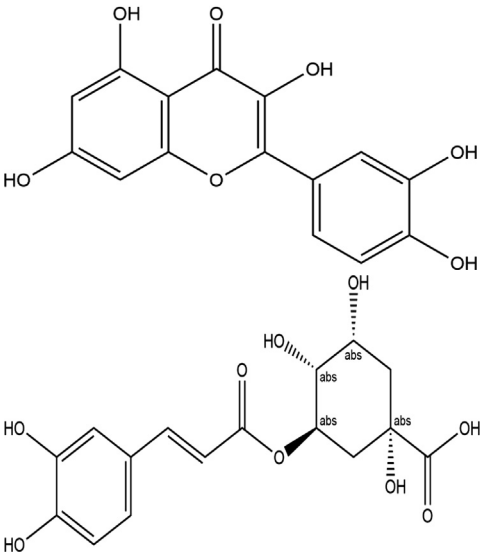
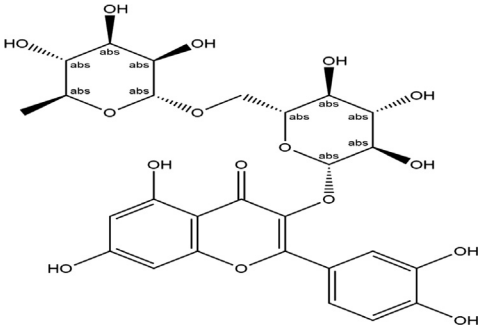
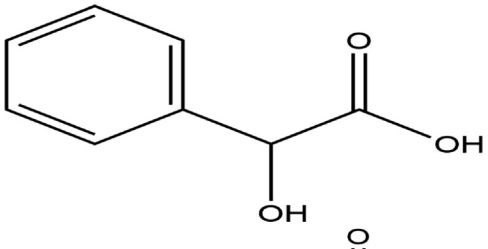
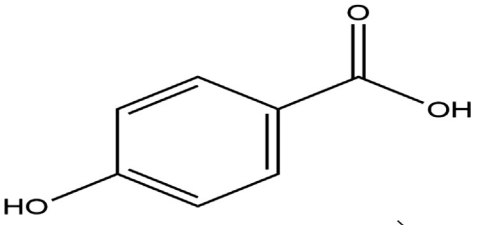
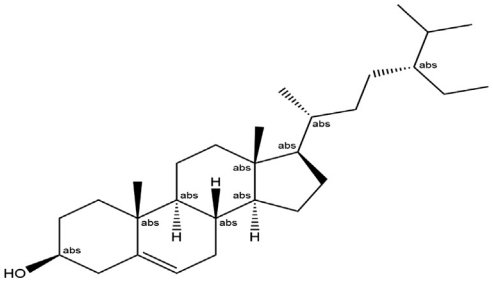
S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
1	***Berberim lyceum***	Berbamunine	440,585		−9.1067	1.9753	ASP125, TRP384
2		Beberine	2353		−6.5408	0.8408	GLY175, GLY174
3		Palamtine	19,009		−7.0056	1.2755	GLY174, GLY175
4		Jatrirrhizine	72,323		−6.9077	1.4044	TYR388
5		4-methyl-7-hydroxycoumarin	5,280,567		−5.2401	1.2906	TRP137, GLY175
6		Stigmasterol	5,280,794		−6.8568	1.0454	GLY339
7		Quercetin	5,280,343		−6.6008	1.1818	TRP137, SER179, GLU255

Table 7 (*continued*)

S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
8		Chlorogenic acid	1,794,427		−6.8114	1.0200	TRP384, TRP137, GLY175, Ser179, GLU255
9		Rutin	5,280,805		−8.7013	2.2268	2TYR178, SER179, VAL331, THR335
10		Mandellic acid	1292		−4.9686	0.8510	TRP137, VAL123
11		Hydroxy benzoic acid	135		−4.5861	1.2320	TRP137, TRP384, GLY180
12		Beta-sitesterol	222,284		−6.8890	4.9210	GLY339
13		Anthocyanin	145,858		−5.7303	0.6397	TRP137

(*continued on next page*)

Table 7 (*continued*)

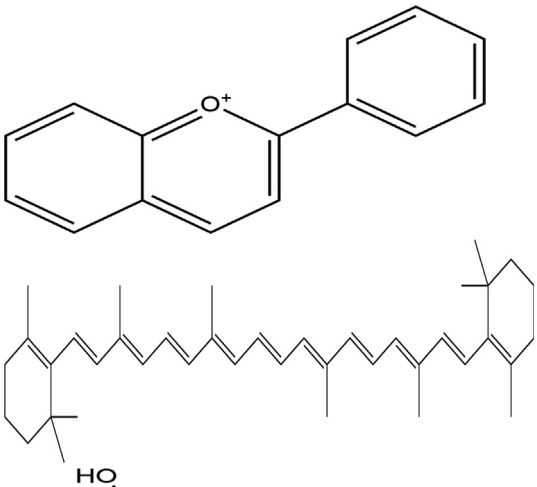
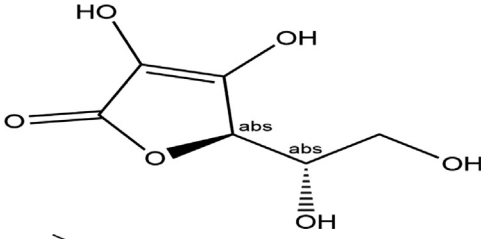
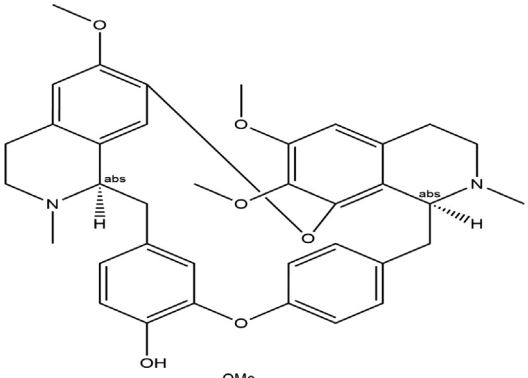
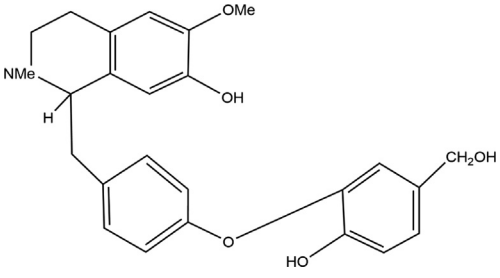
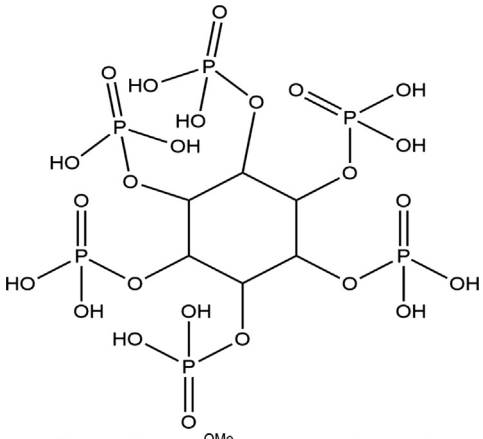
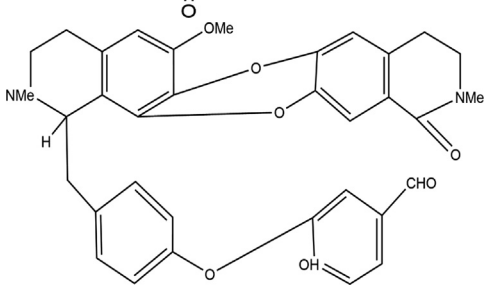
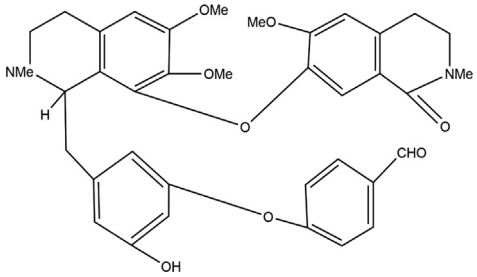
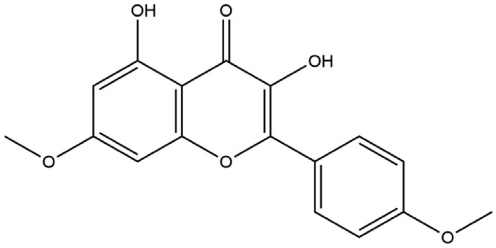
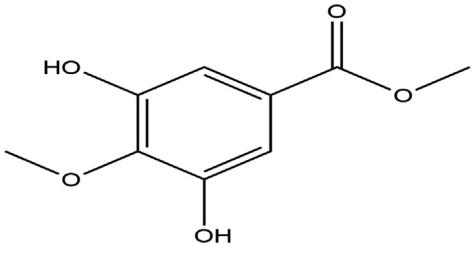
S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
14		Beta-carotene	5,280,489		−7.6714	2.3674	TRP137
15		Ascorbic acid	54,670,067		−5.0701	2.7772	2GLU255
16		Oxyacanthine	442,333		−8.6662	1.3478	PHE343
17		Karakoramine			−6.77787	2.0127	ASP125, THR335

Table 7 (*continued*)

S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
18		Phytic acid	890		−7.0390	1.9271	2TYR178, THR335
19		Punjabine			−7.7278	1.5047	Asp125, PRO344
20		Sindamine			−8.2700	2.1248	LEU124, ASN138
21	***Tamarixaphylla***	Kaempferol-7,4-dimethyl ether	5,378,823		−6.9881	0.7784	TRP137, GLU255
22		Methyl 4-o-methylgallate	5,319,726		−5.2448	0.5589	TRP384

(*continued on next page*)

Table 7 (*continued*)

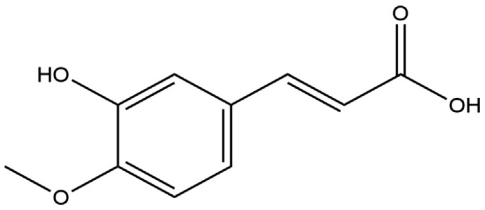
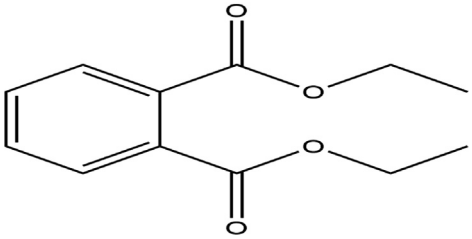
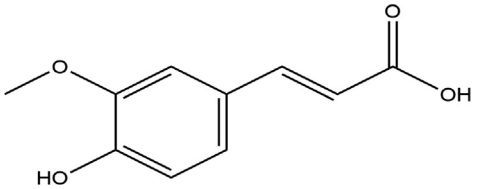
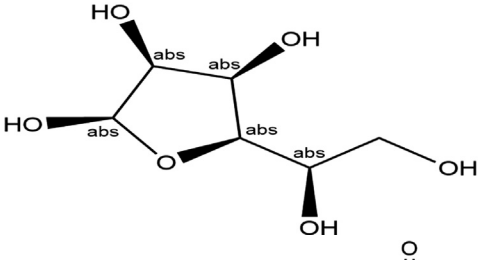
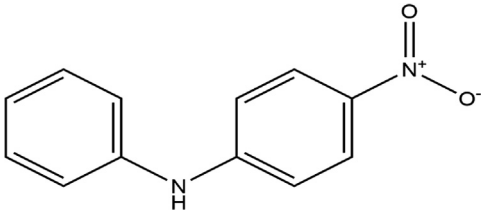
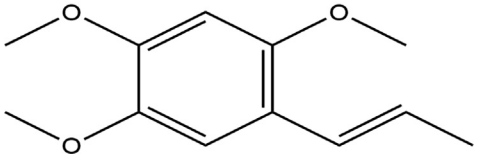
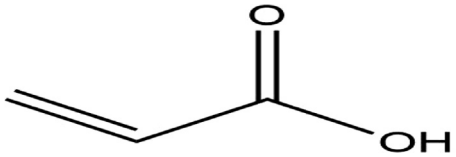
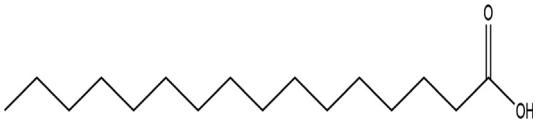
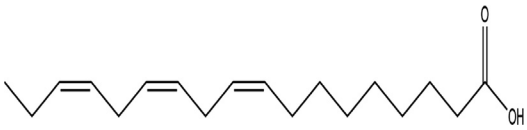
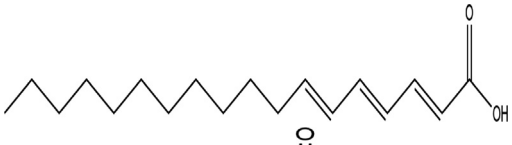
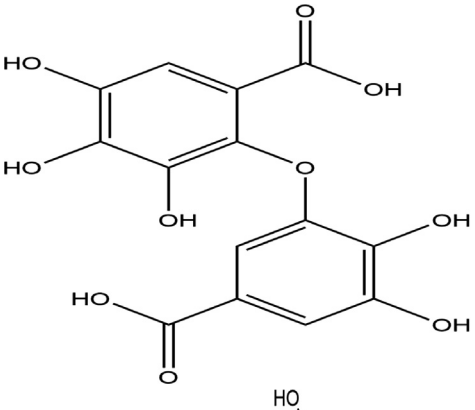
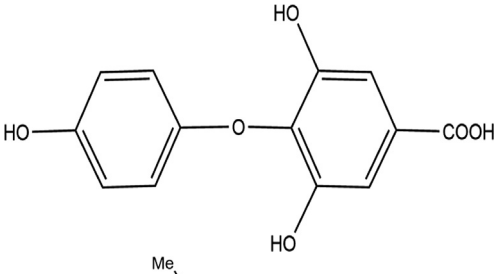
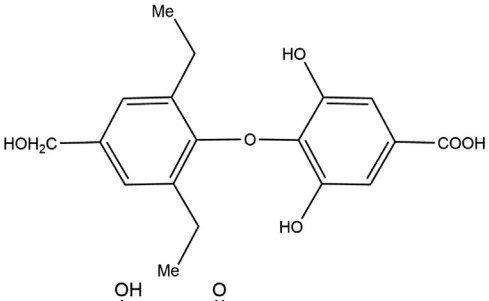
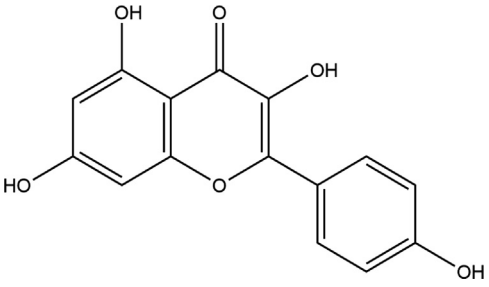
S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
23		Trans-isoferulic acid	736,186		−5.1716	1.5681	GLU255, SER256, TRP384
24		Diethyl phthalate	6781		−5.8530	2.6833	TRP137, TRP384, PHE343
25		Ferulic acid	445,858		−5.1099	1.7039	GLY495
26		Beta-D-mannofuranoe	21,627,869		−5.2992	2.4927	2GLU255
27		Benzenamine, 4-nitro-N-phenyl	13,271		−5.5765	0.5026	SER256
28		Asarone	636,822		−5.8007	1.2521	GLY175
29		Octadecene	8217		−7.1207	0.7759	SER134
30		2-propenoic acid	6581		−3.7076533	0.7232	VAL123
31		n-hexadecanoic acid	985		−6.9606	0.8875	SER134

Table 7 (*continued*)

S. NO	Plant Name	Compound Name	PubChem ID	Compound structures	Docking Score	Rmsd_refine	Amino acids residue interactions
32		Phytol	5,280,435		−7.1680	1.5848	TYR187, GLY180
33		Lenolenic acid	5,280,934		−7.2185	0.8120	VAL123
34		Octadecatrieoi acid	6,506,665		−7.5197	1.0776	SER134
35		Dehydrodigallic acid	14,057,208		−7.1412	1.6211	TRP137, GLY175, GLU255, GLY495
36		Aphylloic acid	–		−5.9521	2.1489	VAL123
37		Tamarixoic acid	–		−7.1330	2.4211	TYR187, GLY180, PHE385, TYR388
38		Kaempferol			−6.4263	1.2592	TRP137, SER179, GLU255

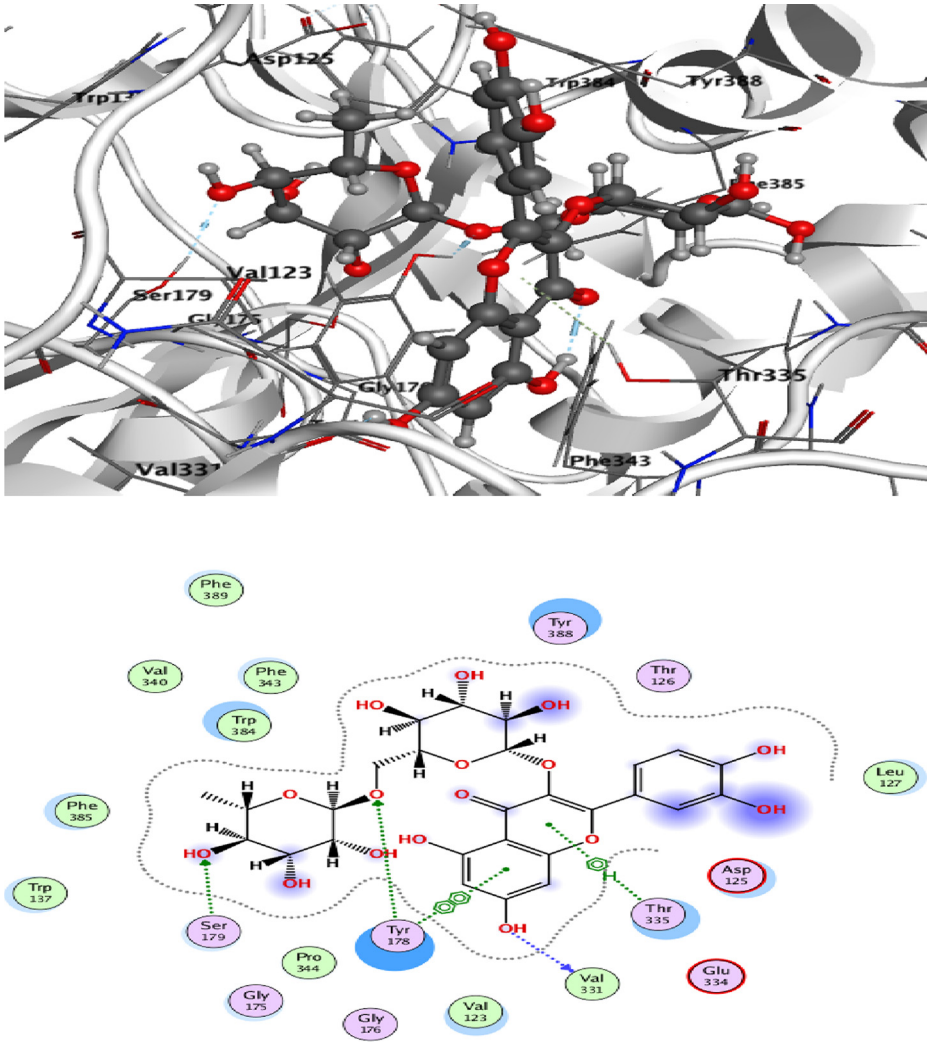


Fig 10. Figure illustrating rutin's 3D and 2D interaction with *R. microplus*'s *AChE1*.

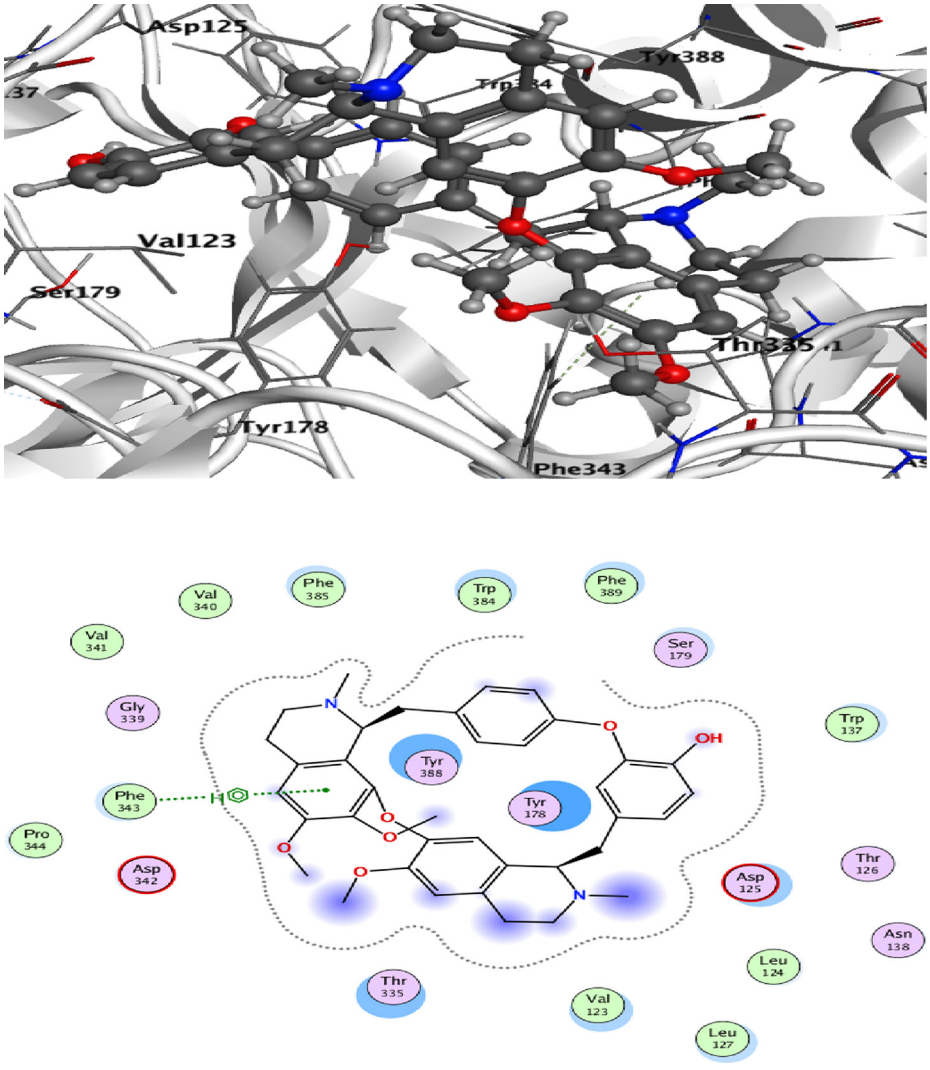


Fig 11. Figure showing Oxyacanthine's 3D and 2D coupling with R. microplus's *AChE1*.

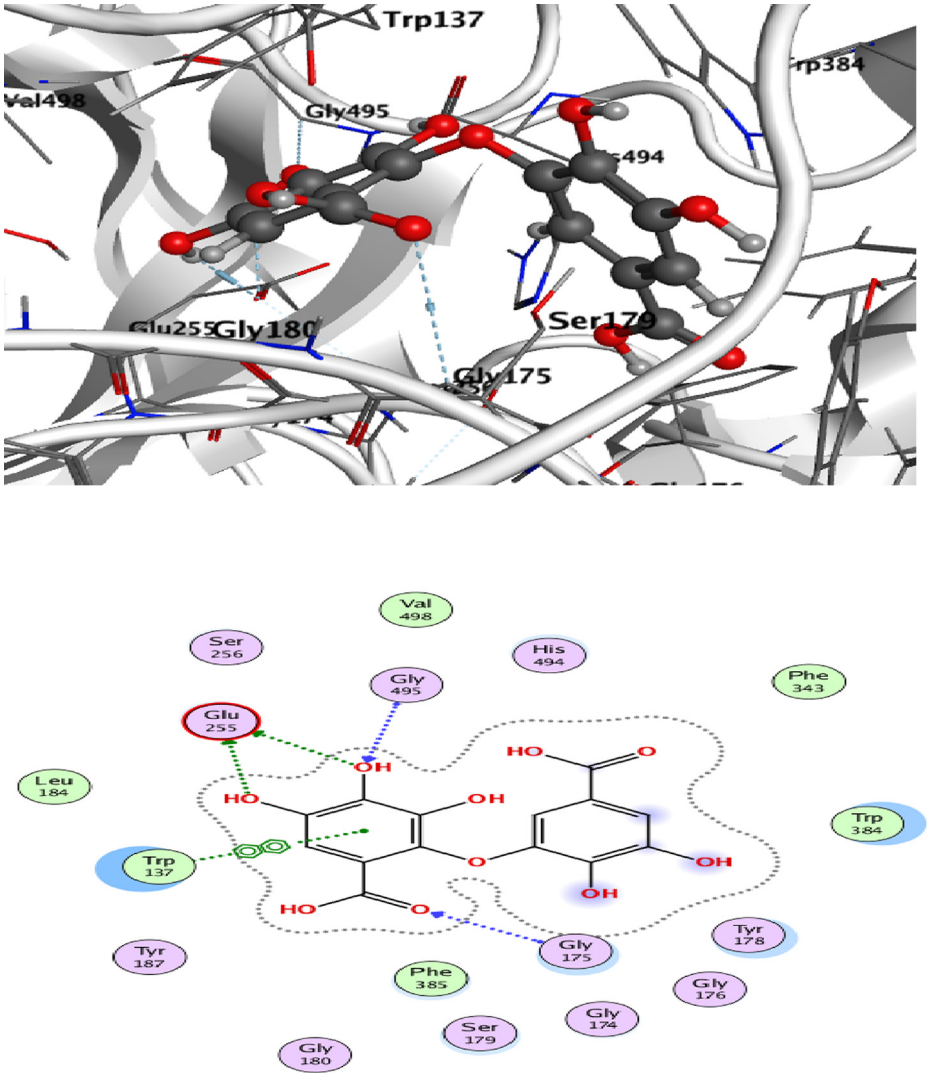


Fig 12. Figure depicting dehydrodigallic acid's 3D and 2D interaction with *R. microplus*'s *AChE1*.

ogy modelling, we suggested the first 3 dimensional structure for *Rhipicephalus microplus* AChE1. We presented the 3 dimensional structure for *Rhipicephalus microplus* AChE1 using different software of homology modelling. The phytochemicals effects on the active sites of the AChE1 model were investigated. Our molecular docking results along with experimental data for *RmAChE1* inhibition showed that activate medicines preferentially interface with the Chain A active sites with exception of berbamunine which interfaces better with the active sites of AChE1. Natural plants containing acetylcholinesterase inhibitors may help reduce ticks and tick-borne illnesses in regional context. Given the current state of acaricide resistance, a comprehensive investigation to identify native species that are effective acaricides should be a high priority of our future research studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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