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Molecular Docking Studies to Validate the Efficacy of the Indigenous Bioformulation of North East India against the Reproductive Proteins of the Pest, *Tetranychus urticae*

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Abstract

Tetranychus urticae (two-spotted spider mite) is a pest infesting multiple crops of agricultural importance. An effective remedy for the prevention and control of this pest is lacking. This study focuses on the preparation and identification of the phytocompounds from the indigenous bioformulation by GC-MS analysis. Further, the identified compounds were targeted against the proteins present in the reproductive cycle of the pest by molecular docking. Molecular docking analysis revealed an effective binding score against the vital proteins which will be effective in arresting the multiplication of the two-spotted spider mites. Since the phytocompound affect the reproductive proteins, its further multiplication and spread in the field can be averted. The current *in silico* study depicts the interaction of the mite protein and the phytocompound which also effective against other pests. This is a first ever report for the characterization of the bioformulation against pests from this region.

***Key words*:** Bioformulation, Molecular docking, Vitellogenesis, *Tetranychus urticae,* Ecdysone, Acaricide

Tetranychus urticae is a polyphagous pest belonging to the family of web-spinning mites, Tetranychidae that causes huge damage to various crop plants [1-2]. It is commonly known as a two-spotted spider mite (TSSM) with a wide host range causing serious economic loss to the farmers. The ability to produce web aids in forming a micro habitat that protects them from abiotic agents and predators thus making the mitigation efforts cumbersome. It can feed about 1200 plant species of which over a hundred are of agricultural importance including soybean, tomatoes, cucumber, cotton, leafy greens, etc. [3-4]. This pest is characterized by rapid multiplication, reduced development time, and a high adult survival rate [5], making it notorious among the pests. Consumption of the host plant by the pest, results in the reduction of leaf surface area for photosynthesis, as they develop chlorotic and necrotic spots after infestation. Furthermore, the injection of phytotoxic chemicals by the mite also alters the biochemical composition of the host plants [6-7]. These factors make it mandatory to control the pests in the interest of farmers as well as consumers.

Synthetic pesticides have been used to control or manage the pest but an excessive amount of synthetic pesticides can cause some adverse effects on crop plants, such as the development of resistance against host defence mechanisms, deleterious effects on non-target organisms, and accumulation of pesticide residue on leaves [8]. Therefore, there is a high demand for ecologically friendly, sustainable control techniques so that farmers can switch from synthetic pesticides to organic pesticides. Pesticides made from plant extracts are the most effective alternative approaches available for reducing insect populations [9]. Allelochemicals, or molecules crucial in plant-insect interactions, found in plant extracts, are increasingly being investigated as a substitute for or addition to synthetic insecticides [10]. These botanicals can be enhanced by the addition of animal products such as cow dung, cow urine etc. Cow and its bye products are an integral part of indigenous agricultural practices in India from time immemorial [11]. The cow urine constitutes various minerals such as sulphur, sodium, nitrogen and other acids and enzymes which has proven insecticidal properties [12-15].

Though various bio formulations are in use, very few attempts have been carried out for the biochemical characterization of these indigenous formulations. The scientific validation of these bioformulations will help in establishing and popularizing these age-old practices [16].

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Hence, in this study, we have tried to identify the phytocompounds of an indigenous anti-pest bioformulation that has been prepared based on the cultural practices prevailing among the tea estates of North Bengal, India by GC-MS analysis. The present study also focuses on testing the efficacy of the bioactive compounds against *Tetranychus urticae* (TSSM). For this, an *in-silico* approach by molecular docking of the phytocompounds against various proteins involved in the reproductive stage of the *Tetranychus urticae* is employed. Our analysis showed the presence of a potential bioactive compound that is effective against almost all mite proteins under study. This particular compound can be targeted for the production of a biopesticide or acaricide which can be of commercial importance. Though TSSM is not common in tea, this bioformulation under study is also very effective for other tea pests such as red spider mite and is widely used by tea garden workers of this region.

MATERIALS AND METHODS

Preparation of bioformulation sample

Bioformulation was prepared based on the indigenous knowledge and the cultural practices of various tea gardens of Dooars region of West Bengal, India. The formulation comprises the aqueous extracts of the plants such as *Melia azedarach* (Ghora neem), *Clerodendrom viscosum* (Ghato), *Vitex negundo* (Nishinda), *Justicia adhatoda* (Basak), *Polygonum hydropiper* (Sukurpota), and non-edible ferns. These plants are widely known for their pesticidal activity and are largely utilized as the part of the integrated pest management. For the preparation of this formulation, the young stems with leaves of each plant 400 gm for 10 L were cut into small pieces, dried under the shade, and then soaked in water. The cow urine 500 mL was added to this mixture as an enhancer and kept it for 10-12 days at room temperature. After the incubation period, the mixture was sieved through muslin cloth and the filtrate was ready to use as a foliar spray in the field.

GC-MS analysis

The samples were prepared for GC-MS analysis by drying the aqueous extract of bioformulation in an oven at a temperature of 40°C for 3 days. The dried extract was dissolved in methanol and the methanolic extract was sent for GC-MS analysis. Analysis was performed in AIRF-JNU employing a GCMS-QP2010 ultra (Shimadzu Corporation, Kyoto Japan). Here, an Rtx 5 MS capillary column was used to separate the chemicals present in the solvent extracts (Restek Company, Bellefonte, USA). The split mode was employed at a 10:0 ratio. The temperature of the injector in split injection mode was set to 250°C (hold time 7 mins) which was gradually ramped up to 280°C (hold time 13 mins) at a rate of 15°C/min after being configured to start at 100°C for 3 minutes hold time. Helium was used as a carrier gas with a linear flow velocity of 40.9 cm/s. A total of 1µl of the solvent extract was administered and the components present in the extract were identified by comparing their retention indices (RI) to homologous alkane series, as well as their mass spectral fragmentation patterns to data from libraries such as NIST.LIB, NIST08.LIB, NIST08s.LIB, and WILEY8.LIB. The assumption or identification of the compounds is based on a good match between the mass spectrum and the RI.

Retrieval of protein sequence from NCBI

The amino acid sequences of different proteins involved in the reproductive system of the pest *Tetranychus urticae* were retrieved from NCBI in the faa format. A total of 20 proteins are retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/).

Building a protein model and ligand structure

After retrieving the protein sequences, their 3D structures were built in the Swiss model server (https://swissmodel.expasy.org) [17] and the models were saved in PDB format. The compounds identified from the bioformulation after GC-MS analysis is provided in the supplementary table. The three-dimensional structures were downloaded from Pubchem in the SDF format and the ligand was prepared using AutoDock vina [18-19]. The target protein was prepared for docking analysis by eliminating the water molecules and polar hydrogen atoms and Kollman charges were added to the structures using AutoDock software.

Molecular docking

After building a model, blind molecular docking was done by PyRx software [20]. The prepared ligand was docked against the 20 different proteins involved in the reproductive and developmental stages of *T. urticae*. We obtained nine configurations for each target protein after each blind docking. Based on the binding score, we chose the first configuration out of the group.

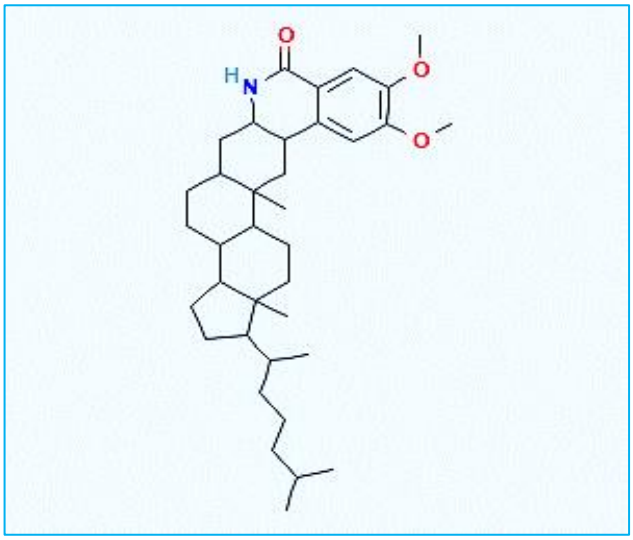

Fig 1 Phytocompound 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A structure

RESULTS AND DISCUSSION

The female spider mite form webs on the leaf surface and lays eggs thereby protecting the eggs from predators. Moreover, the larvae feed on the leaves and grow into adults under maternal care [21]. The lifecycle of the spider mite consists of egg, larvae, nymph 1st stage, nymph 2nd stage, and adult. The targeted proteins were identified from vital stages such as vitellogenesis, ecdysone synthesis, and oviposition. Docking was conducted with the proteins involved in the reproduction of *T. urticae* with the ligand1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A (Fig 1) (Pub ID: 541426). This compound from the bio-formulation showed the interaction with the Vitellogenesis proteins and binding energy scores obtained was -9.7 kcal/mol. Further, the phytocompunds interacted with molting proteins with a binding energy score in the range of -8.7 to -12 kcal/mol. The binding energy score of the proteins with the phytocompound 1-(1,5-Dimethylhexyl)-10,11-Dimethoxy-13A,15A and the interacting amino acid residues are provided in (Table 1). The studied phytocompound

proved its efficacy in arresting the reproductive cycle and helping in reducing the population of the voracious mite. The action of the phytocompound in the cardinal stages of the lifecycle and the responsible genes are depicted in (Fig 2).

Table 1 Interaction profile of the phytocompounds against TSSM reproductive proteins

Protein name	Locus tag	Docking score (kcal/mol)	Interacting residues
Vitellogenins and vitellogenesis			
Vitellogenin receptor	XP_015784256.2	-9.7	*Ile527, Ile567, Thr529, Glu512, Arg510, Leu517, Asn515, Phe516, Val566, Leu565*
Follistatin-related protein 5-like	XP_015786878.1	-9.7	Ser392, *Ala423, Leu424, Arg425, Trp431*
Sex peptide receptor			
Thyrotropin-releasing hormone receptor-like	XP_015783705.1	-10.9	*Ala226, Lys222, Trp192, Val227, Phe293, Val195, Thr196, Val223, Arg219*
Ecdysone synthesis and regulation			
Ecdysone 20-monooxygenase	XP_025015989.1	-12	Asn74, *Asn95, Leu77, Gly292, Val291, Asn295, Phe466, Phe194, Ser94, Phe197*
Diuretic hormone receptor-like	XP_015782298.1	-11.2	*Leu354, Phe216, Leu302, Tyr350, Thr212, Trp180, Tyr294, Trp284, Pro283, Asn291, Val286, Trp259, Ile295*
Gonadotropin-releasing hormone receptor	XP_015783441.1	-11.2	*Ile98, Thr95, Arg122, Glu99, Gln119, Phe319, Gln209, Gln298, Val294, Phe297, Met312, Ser195, Phe179, Cys193, Trp108*
Hepatocyte growth factor receptor	XP_015781909.1	-11	*Phe272, Gly263, Ile261, Gly196, Asn197, Tyr262, Leu74, Ala488, Leu72, Gly487, Pro486, Lys71, Ala195, Phe272*
Ecdysone-induced protein 78C isoform X4	XP_015784375.1	-10.8	*Pro209, Phe261, Phe206, Thr260, Phe203, Arg202, Asp205*
Fibroblast growth factor receptor 1-like	XP_015781782.1	-10.7	*Tyr82, Gln80, Pro70, Leu181, Leu183, Thr180, Arg69, Ser207, Phe86, Phe179, Lys208, Leu81, Pro83*
Ecdysone receptor	XP_015787529.1	-10.4	*Gly400, Glu280, Ser276, Ile424, Lys275, Gln353, Pro352, Arg351, Arg397*
Nuclear hormone receptor E75	XP_015789751.1	-9.9	*Ile223, Val227, Lys231, Ala237, Gln244, Gln241, Leu248, Val245, Ile223*
Protein tailless-like	XP_015785154.1	-9.1	*Lys85, Asp45, Arg46, Tyr48, Thr49, Cys50, Asn58, Arg76, Leu75, Ala72, Ala79, Ile42*
Probable nuclear hormone receptor HR3	XP_015781040.1	-8.7	Thr387, Lys512, *Glu391, Glu463, Phe457*
Oviposition			
Aromatic L amino acid decarboxylase like	XP_015784000	-13.2	Phe80, *Val101, Lys302, Phe103, Leu350, Pro434, Cys435, Ser433, Tyr79, Thr245, His191*
Neuropeptide F receptor like	XP_015795984.1	-12.2	Thr237, *Leu236, Trp240, Phe123, Ile92, Asn85, Leu88*
Calcium calmodulin-dependent protein kinase type II alpha chain	XP_025016044.1	-12.1	*Pro234, Tyr211, Lys293, Pro212, Arg297, Trp215, Phe294, Asp232*
Synaptic vesicular amine transporter	XP_015783060.1	-11.3	*Leu309, Phe306, Arg302, Tyr297, Gly293, Pro305, Met296, Phe292*
Dopamine beta-hydroxylase-like	XP_015783011.1	-11	*Arg328, Val395, Tyr522, His443, Gln476, Gln441, Val329, Ser327*
Octopamine receptor beta-2R-like	XP_015785358.1	-10.7	*Val235, Ala321, Leu320, Trp324, Leu247, Phe282, Phe251, Thr328*
Clathrin heavy chain 1	XP_015788618.1	-10.1	*Thr1383, Thr1414, Trp1386, Lys1415, Tyr1411, Pro1382*

Hydrogen bonding is represented in bold text while Hydrophobic bonding are represented by italicized text

The total list of phytochemicals obtained from the bioformulations after GC MS analysis

Peak	Retention time	Area	Area%	Name
1	4.109	398671	0.81	CYCLOHEXANE, 1-ETHYL-2-METHYL-, TRANS-
2	4.515	1058723	2.14	2,4,4-Trimethyl-1-hexene
3	4.635	480175	0.97	2,4,4-Trimethyl-1-hexene
4	4.783	199061	0.40	Cyclohexane, 1-isopropyl-1-methyl-
5	4.911	478561	0.97	N-ETHYL,N-VINYLACETAMIDE
6	5.717	689470	1.40	ACETAMIDE, N,N-DIETHYL-
7	5.959	311982	0.63	N,N,2-TRIMETHYLPROPANAMIDE
8	8.100	41574569	84.17	Trifluoroacetic acid

9	9.295	970129	1.96	1-Propanamine, N-ethyl-N-methyl-
10	12.447	40576	0.08	Sulfurous acid, hexyl octyl ester
11	13.123	1008126	2.04	PHENOL, 2,6-BIS(1,1-DIMETHYLETHYL)-
12	13.871	305588	0.62	Tetradecane
13	14.962	67183	0.14	Nonane, 5-(2-methylpropyl)-
14	15.909	66096	0.13	BIS(TRIMETHYLSILYL)-2-[HYDROXY-TRIS(ETHOXY
15	16.108	562581	1.14	NONADECANE
16	18.148	435243	0.88	OCTADECANE
17	19.230	205970	0.42	Nonyl tetradecyl ether
18	19.700	27592	0.06	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-3,4
19	20.005	224409	0.45	Heneicosane
20	20.894	62951	0.13	1-(1,5-DIMETHYLHEXYL)-10,11-DIMETHOXY-13A,15
21	21.078	76639	0.16	Dodecane
22	21.769	149460	0.30	Pentadecane, 8-hexyl-

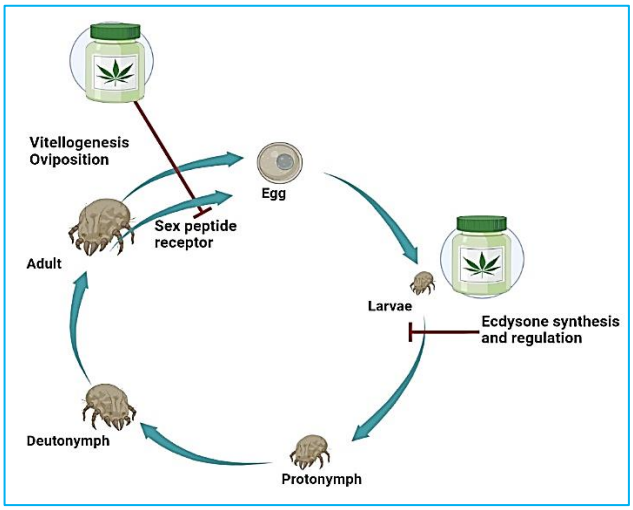

Fig 2 Life cycle of two spotted red spider mite interacting with phytocompounds

Docking with proteins of sex peptide receptor

The chosen protein, the Sex peptide receptor is involved in the release of stored sperms for fertilization [22]. Our ligand 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A from the bio formulation showed the binding score of -10.9Kcal/mol with the sex peptide receptor. The residues in the positions Ala226, Lys222, Trp192, Val227, Phe293, Val195, Thr196, Val223, and Arg219 were found to be interacting with the ligand with high efficiency (Fig 3). Since this protein is crucial for the fertilization process, blocking this protein will be an ideal strategy for pest management.

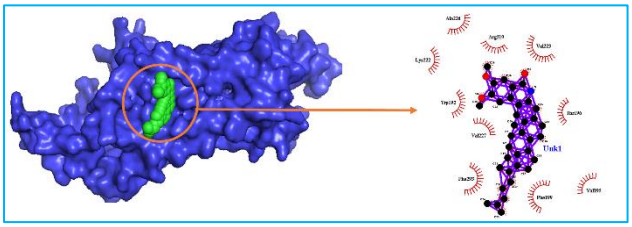

Fig 3 Mode of interaction of the thyrotropin-releasing hormone receptor-like. Protein with 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A

Docking with proteins of vitellogenesis

Vitellogenesis is a vital step in the yolk formation of the eggs that involves the synthesis and storage of carbohydrates, lipids, and proteins [23]. The ligand 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A showed a binding energy score of -9.7 Kcal/mol with the vitellogenin receptor. The interacting residues found to be Ile527, Ile567, Thr529, Glu512, Arg510, Leu517, Asn515, Phe516, Val566, and Leu565 (Fig 4). The formation of eggs is blocked which arrests the further proliferation of the mite.

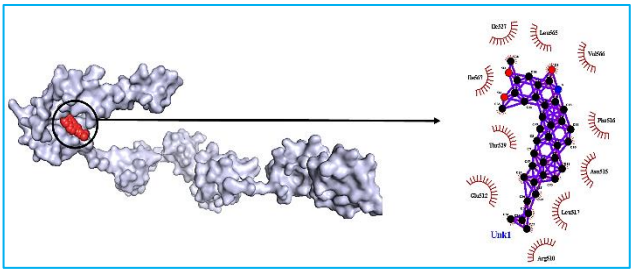

Fig 4 Mode of interaction of the vitellogenin receptor Proteins with 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A

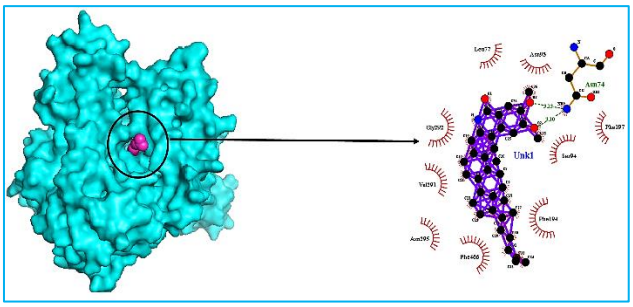

Fig 5 Mode of interaction of the aromatic L amino acid decarboxylase like protein with 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A

Docking with proteins of oviposition

The proteins involved in the laying and expulsion of eggs are the oviposition proteins. The ligand under study, 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A showed the binding score of -13.2 Kcal/mol with the Oviposition proteins receptor. The interacting residues found to be Phe80**,** Val101, Lys302, Phe103, Leu350, Pro434, Cys435, Ser433, Tyr79, Thr245, and His191 (Fig 5). The spider mite has a defensive mechanism to protect their eggs from predators by laying eggs on the webs produced on the leaf surface [24]. Though this defense mechanism can deter the predators, this phytocompound can efficiently arrest the entire process of oviposition of mite. This invariably shows the efficacy of this phytocompound in arresting the reproductive process of the TSSM where the biological control measures fail to give success.

Docking with proteins of ecdysone proteins

Ecdysone synthesis is crucial for the molting and metamorphosis of larval forms to adults [25]. The ligand 1-(1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A showed the binding score of -12 Kcal/mol with the ecdysone proteins

receptor, thereby terminating the lifecycle of the *Tetranychus urticae*. The interacting residues were in the positions Asn74, Asn95, Leu77, Gly292, Val291, Asn295, Phe466, Phe194, Ser94, Phe197 (Fig 6).

Though the above-mentioned compound was reported from the leaves and stems of *Clematis graveolens*, of the family Ranunculaceae there is no further evidence about the functional aspect of this phytochemical. Our study provides substantial evidence for the pesticide activity of this phytocompound and its inhibitory activity against the proteins involved in the reproductive and developmental stages of *Tetranychus urticae*. This study will be a pioneer work to show the *in-silico* evidence for the interactions of this compound against the reproductive proteins of the spider mite.

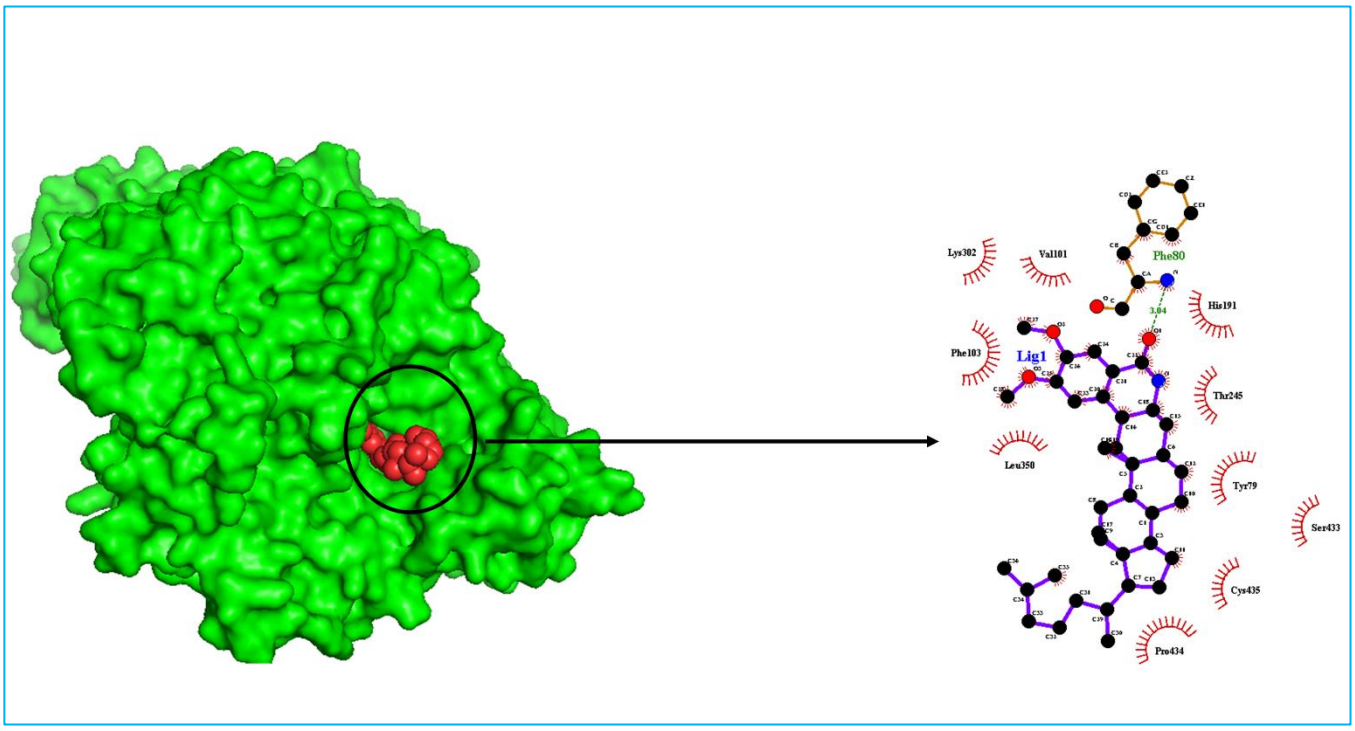

Fig 6 Mode of interaction of the ecdysone 20-monooxygenase Proteins with - (1, 5-Dimethylhexyl)-10, 11-Dimethoxy-13A, 15A

CONCLUSION

The *T. urticae* (TSSM) is a rapidly multiplying tropical pests that complete their lifecycle in 8-12 days. The adult female shows diapause and can lay 90 to 110 eggs which develop in the protective web spinned by the mother mite. Though natural predators are available, most of them are localized and noneffective in other regions; so it is difficult to control them economically. Early detection and prevention are the golden rule in pest management. However, the infestation of the two-spotted spider mites becomes evident in much later stages when the leaves of the crops are already affected. Another blockade to the control measure of this pest is its efficiency to detoxify the chemicals targeted for them. These factors make it very difficult to control this mite which causes heavy loss to the crop plants. It has become mandatory to use target-specific acaricide for its effective control. This phytocompound from the bioformulation showed effective binding with the studied proteins involved in the reproductive cycle thereby preventing its multiplication process. Moreover, the studied bioformulation display significant acaricidal activity against the red spider mite of the tea plantation of the North Bengal region. The identified compound can be exploited commercially as acaricide for the prevention and control of various mites which infest the crop plants.

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