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Isolation of a major host plant-terpenoid and its probable impact on the degenerative growth of female Indian lac insect, *Kerria lacca* (Kerr.)

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Abstract
The present work has been attempted to work out the isolation and characterization of a terpenoid from a major host plant *Butea monosperma* of Indian lac insect (*Kerria lacca*), and its probable impact on the degenerative growth pattern of the female morph, which losses its legs and antennae in its adult stage. The studied terpenoid ($C_{30}H_{52}O$) as ingested from the host plant by the female *K. lacca* probably plays a key role by inhibiting the acetylcholinesterase (AChE, EC 3.1.1.7) undergoing the process of uncompetitive inhibition. This type of inhibition is supported by bio-informatics studies also. This inhibition of AChE may be one of the responsible factors for the degenerative growth pattern of female *K. lacca* featuring the loss of all locomotory organs (legs) and antenna, *etc.* This is most probably caused by the rapid deacceleration of the molar hydrolysis of substrate acetylcholine by the enzyme AChE under such uncompetitive inhibition by the terpenoid as studied.

Keywords: *Butea monosperma*, *Kerria lacca*, terpenoid, acetylcholinesterase, uncompetitive inhibition

Introduction
Most intriguing domain of insect-plant interactions is selection of plants as host, and adaptation of insects against the detrimental secondary plant metabolites on which they thrive [1, 2]. Host plants produce several secondary metabolites (alkaloids, phenolics and terpenoids) to resist against their herbivorous pests [3]. Few plant secondary metabolites, such as allelochemicals, have been known to inhibit the growth parameters of its target pest organisms by several ways, such as by inhibition of neurotransmitters, ion channel receptors and respiration pathway, *etc.* [4-9]. Insect defense strategies against the plant secondary metabolites in their food items are also predisposed by several detoxifying enzymes belonging to Cytochrome P450 family [10, 11]. In the present study the Indian lac insect *Kerria lacca*, has been selected as a model insect to understand the effect of host plant terpenoids on its growth pattern, especially of the female morph. Indian lac insect, *K. lacca* belongs to the Order- Hemiptera, Family- Kerriidae. Insects of this order are characterized by modified mouth parts known as rostrum. The mouth parts are modified typically for piercing and sucking the plant tissue-sap [12]. The crawling nymphal stages of these insect, within a few hours of its emergences from the encrustation shell of mature female lac insects, are able to successfully penetrate the host plant tissues in order to access the phloem tissue sap [13]. Unlike other Homopteran, the lac insect, once inserts its proboscis, remains sedentary for the rest of life [14]. During this phase the insect starts secreting lac, the only known resin of animal origin [12]. Encrustation of lac resin is essential to minimize their chances of predations since they have a very vulnerable sedentary life [14, 15]. Among different species of host plants, the large scale production of lac in West Bengal, India, depends mostly on three plants viz. *Butea monosperma*, *Ziziphus mauritiana* and *Schlischeria oleosa* [12, 14, 16]. Like all hemi-metabolous insect species, *K. lacca* passes through different nymphal stages during the courses of its post-embryonic development [14]. In early nymphal stages it is ovate in out-line, slightly pointed posteriorly as well as soft, crimson colored body with one pair of antennae and three pairs of thoracic legs [14].

But in the case of female morph during its post-embryonic development it loses all types of effective organs, like legs, antennae, wings, *etc*. and becomes pyriform to globular in shape, which is not a usual trait in case of adult males [14]. Several terpenoids of host plants are known to be potent inhibitors of Acetylcholinesterase (AChE) of their pestiferous organisms thriving on them [9]. The principal objectives of the present study are isolation, characterization, and to ascertain the role of host plant terpenoids on the retrogressive growth of female morph of *K. lacca*, if any.

Materials and Methods

- **Study periods**

The entire study was conducted between the months of March, 2013 to December, 2015. Bark of the host plant was collected after sixth months from the date of pruning of host plants (pruning of plant twigs is a common practice in lac culture in India to make the host plants receptive to the *K. lacca* nymphs) in order to isolate and purify the inhibitor terpenoid molecule of AChE.

- **Study area**

Necessary field works and sample collections were carried out at two different Brood lac Culture Farms, Gananondo Saraswati Ashram, Dalpur ($23^0 20' 17.19''$N, $86^0 54' 32.44''$E) Block-Chhatna and Tarkajor Brood Lac Culture Farm, Sunukpahari ($23^0 09' 25.33''$N, $86^0 59' 01.04''$E) Block-Bankura I, at district Bankura, West Bengal, India.

- **Study specimen**

Both nymphal, and adult female morphs of *Kerria lacca* were collected from host plant *B. monosperma* in order to study the specific activity of AChE following the protocol of Ellman *et al* [17].

In order to have a standard Michel-Mention (MM) plot and Lineweaver-Burk (LB) plot of AChE kinetics in the presence of terpenoid inhibitor *in vitro*, pure strain of adult *Drosophila melanogaster* was collected from the Laboratory of Molecular Biology and Cytogenetic, Department of Zoology, Burdwan University, Burdwan, West Bengal, India.

- **Specific activity study on AChE**

The specific activity (molar hydrolysis rate of substrate/min./mg of protein) of enzyme kinetics study of Acetylcholinesterase was done following the protocol of Ellman *et al* [17]. DTNB (10mmol/lit), Sodium phosphate buffer (100mmol/lit at pH 7.0 and 8.0), Sodium bicarbonate (1.5mg/ml), Acetylthiocholine iodide (10mg/ml) were used as reagent stocks. Substrate was prepared with double distilled water and DTNB was maintained at Sodium phosphate buffer (at pH 7.0) with Sodium bicarbonate.

The equation followed:

Specific activity (Rs) = [A] × [total volume in cuvette (µl)] / [Molar extinction coefficient in DTNB] × [volume of brain homogenate µl] × [protein concentration mg/ml]

Where: -

Rs = moles of substrate hydrolyzed / minute / mg of protein

A = change in O.D. per minute = Slope

Molar extinction coefficient of DTNB = 1.36×10^4

Volume of 2% Brain homogenate = 20µl

Volume in Cuvette = 20ul [vol. of 2% brain homogenate] + 151ul [vol. of phosphate buffer pH 8.0] + 131ul [vol. of phosphate buffer pH 7.0] + 5ul of DTNB + 1ul of Acetylthiocholine Iodide =308µl

- **Preparation of plant extract and purification and isolation of single compound from a terpenoids moiety**

Isolated bark sample from host plant *B. monosperma* was air dried and crushed with mixture grinder. Solvent chloroform was used for the purpose of phytochemical extraction in soxhlator. Soxhlated phytochemical was concentrated from solvent $CHCl_3$ using the rotary evaporator (Buchi, model no. R3). Column chromatography ($CHCl_3$: Ethyl Acetate = 8: 1) was performed using Silica gel (mesh, 230-400) to purify it. Activated charcoal was used to remove the necessary pigments from column derived products. Targeted phytochemicals were fully purified after performing the preparative thin layer chromatography (Silica Gel- GF 254,). Three different solvent systems had been used at preparative thin layer chromatography by following the same method, *viz*. Chloroform: Petroleum-Ether (5: 1), Chloroform: Di-Ethyl Ether: Pet Ether (10:2:1) and Chloroform: Ethyl Acetate (8: 1) respectively.

- **Structure analysis of purified phytochemicals**

Qualitative analysis

Necessary qualitative analysis of alkaloids, phenolics and terpenoids were done following the protocol of Pushkar *et al.* [12].

GC-MS Analysis

TG-5MS (5% Phenyl Methylpolysiloxae) column (30 m length, 0.25 mm diameter and 0.25 µm film thickness) was used for gas chromatography with the Helium (He) gas (flow rate 1ml/min) as mobile phase (Thermo Scientific, model name- Trace 1300/Gas Chromatography). Instrumental specification of mass spectrophotometer was Thermo Scientific Trace 1300 GC equipped with ISQ MS and an AI/AS 1310 Auto-sampler. During GC-MS analysis the initial temperature of the column was set at 150 ^{0}C up-to 240 ^{0}C at the acceleration rate of 20 ^{0}C/min and then increased up-to 310 ^{0}C at the rate of 5 ^{0}C/min. Injection temperature was set at 250 °C at a pressure of 5 kPa, with total flow and column flow of 10 ml/min and 1 ml/min, respectively, with a solvent cut time of 5 min. The MS programme starting time was 5 min which ended at 18 min with event time of 0.5 s and mass range 50 to 650. Total run time was 18 minutes. Ion source temperature of Mass- Spectrophotometer was set at 320 ^{0}C. Sample was loaded 1µl (split ratio 10:1) in each and the concentrations were 1mg/ml. HPLC grade hexane was used for dilution and injections of samples.

Infra-Red spectroscopic analysis

FT-IR data were taken using KBr pellet against the KBr blank. Instrumental specification of IR was Perkin-Elmer, Spectrum Two.

^{13}C NMR spectroscopy

$CDCl_3$ solvent was used to dissolve the 20mg of compounds for recording the ^{13}C-NMR (Brucker, 300MHz).

- **Study on Inhibition Kinetics of Acetylcholinesterase**

Spectroscopic study of enzyme inhibition

Inhibition study of AChE was done on the enzyme isolated from normal strain of adult fruit flies, *Drosophila melanogaster*. In order to have a standard Michel-Mention (MM) plot and Lineweaver-Burk (LB) plot of AChE kinetics in the presence of terpenoid inhibitor *in vitro*, total thirty samples of adult fruit flies were homogenized in 1ml of

phosphate buffer (pH 8.0) at 4 ^{0}C temperature. To collect the supernatant the sample was centrifuged at 10000 rpm for next 10 minutes and the temperature was set at 4 ^{0}C.

The purified triterpenoid compound, extracted from host plant *B. monosperma* was used as inhibitor of AChE. 30mg inhibitor was dissolved in 10 ml Methanol (Sigma-Aldrich, molecular biology grade) to prepare the stock of inhibitor. From the stock of inhibitor, 9µl was used in each reaction mixture. In case of control 9 µl of methanol was added to minimize the error. All necessary spectroscopic analysis had done followed by Ellman *et al.* [17]. A stock of the substrate, Acetylthiocholine iodide was prepared 1.5 ml (10mg/ml). To analyze the maximum velocity of reaction (V_{max}) of kinetics study, different concentration of substrate was used from the stock after the necessary serial dilution. Concentration of enzyme, inhibitor and DTNB were constant in each reaction mixture. Maximum velocity of reaction (V_{max}) and Michel-Mention Constant (K_m) value and the type of inhibition were analyzed after the transformation of MM plot into LB plot.

Understanding Inhibition of Acetylcholinesterase (AChE) from bio-informatics approach

The crystal structure of Torpedo acetylcholine esterase (AChE) complexed with acetylthiocholine was retrieved from protein data bank (PDB ID: 2C4H). The two-dimensional (2D) chemical structure of terpenoid was sketched using Chemdraw and converted into the corresponding standard three-dimensional (3D) structure by using *Discovery Studio3.5*. The possible binding sites of terpenoid on the enzyme-substrate complex were identified based on the peripheral anionic site (PAS) that lies essentially on the surface of AChE, approximately 20Å distant from the active site itself [18]. The PAS consists of 5 residues (Tyr70, Asp72, Tyr121, Trp279 and Tyr334) clustered around the entrance to the active site gorge [18]. Before docking of the inhibitor to the enzyme-substrate complex, the crystal structure of AChE complexed with acetylthiocholine and the inhibitor terpenoid were separately minimized energy using CHARMM force field. Docking was carried out with LibDock program. The possible conformations of the studied triterpenoid were generated using BEST algorithm of LibDock. The 60 top-ranked LibDock score were selected for calculation of binding energy between the receptor and a ligand. In situ ligand-receptor minimization was performed to remove any receptor-ligand van der Waal's clashes prior to calculating the binding energy. 1000 steps of Smart Minimizer with free movement of atoms within the binding site sphere were used during the minimization. The lowest binding energy was selected for further calculations and analyses.

Results and Discussions

- **Isolation and characterization of terpenoid from a major host plant *Butea monosperma***

The molecular mass of the isolated compound (Fig. 1) has been determined by EI-MS as 428.96 (Calculated – 428.7398 gm/mol). Based on the m/z values obtained from the EI-MS analysis, the molecular formula $C_{30}H_{52}O$ has been worked-out, which is also confirmed by the ^{13}C NMR spectrum. Several notable fragment peaks have been observed at m/z 57.07 (OH_5C_3), 97.1 ($H_{13}C_7$), 111.16 ($H_{15}C_8$ / $OH_{11}C_7$), 153.24 ($OH_{17}C_{10}$), 181.28 ($OH_{21}C_{12}$) (Fig.2), which are also in conformity with the molecular formula as worked-out $C_{30}H_{52}O$. The IR spectrum has shown a broad band around, 3435.94cm^{-1} clearly indicates the presence of a hydroxyl group (-OH). Other values in IR spectrum at 2917.63 cm^{-1} (C-H stretching), 1637.76 cm^{-1}(C=C stretching) and 1462.76 cm^{-1} (α-CH2 bending) are also in agreement with the structure of the compound worked-out. The ^{13}C NMR spectrum accounts for all 30 Carbons involving five CH_3, fifteen CH_2, five CH and five quaternary carbon atoms. Notably all five CH_3 atoms are attached with quaternary carbon atoms. Apart from IR spectrum, presence of C=C is further supported by the peaks at δ139.28 and 114.06 in ^{13}C-NMR spectrum. Another signal at δ 63.11 represents the presence of $–CH_2OH$ [21]. The presence of methylene carbon ($–CH_2OH$) is further supported by the appearance of negative peak at δ 63.11 in DEPT-135. More over qualitative analysis following the protocol of Pushkar *et al.* [12] confirms that the described compound is a terpenoid. All the spectral analyses including IR, NMR, Mass spectra suggest that the compound which is detected at R_t 13.11min.at Gas chromatogram (Fig. 1.B), consists of six isoprene unit, which also speaks in support of triterpenoid structure. The structural arrangements of isoprene units are as follows: the first three isoprene units are attached following head to tail direction though the first carbon atom of fourth isoprene unit is joined with the third carbon atom of third isoprene unit and the second carbon atom of fourth isoprene unit is connected with the fifth carbon of third isoprene unit. Hydroxyl (–OH) group is attached with the third carbon atom of second isoprene unit. Second carbon atom of fifth unit is also attached with the first carbon atom of fourth isoprene unit. So, it is tenable that the analysis supports towards the arrangement of first five isoprene units in cyclic order. Although fifth and sixth isoprene unit are joined together by tail to tail combination which could be arranged in acyclic form also (Fig. 3).

AChE inhibition Scenario in lac insect

To understand the possible causes of the loss of locomotory activities as well as organs (legs) and antenna after penetrating the rostrum into host tissues, the specific activity (molar hydrolysis rate of substrate/min/mg of protein) of enzyme acetylcholinesterase was measured phase-wise *viz.* nymphal crawling stages and female adult stages. Data obtained from the specific activity of AChE reveals that the rate of substrate hydrolysis gradually decreases with the gradual advancement of growth in due course of time from early nymph to adult female (Fig. 4). It is indicative that due to the inhibition of AChE, the molar hydrolysis rate of substrate acetylcholine decreases rapidly. AChE promotes the hydrolysis of Acetylcholine by forming an acetyl-AChE intermediate with the release of Choline and the subsequent hydrolysis of the intermediates to release acetate [20]. Probable cause of rapid deacceleration of Molar hydrolysis rate of substrate, acetylcholine after penetrating the rostrum of *K. lacca* into the bark of host plant is the AChE inhibition due to the presence of an inhibitor molecule among host plant tissue.

Confirmative kinetics study on enzyme AChE inhibition by the isolated terpenoid in *Drosophila* model

Present study reveals that in the presence of isolated triterpenoid compound from the bark of host plant *B. monosperma*, the maximum velocity (V_{max}) and Michelis-Menten Constant (K_m) values of AChE (enzyme from *D. melanogaster*) kinetics become altered significantly (Table 1). In the present study of kinetics, V_{max} decreases and K_m value increases (Table. 1, Fig. 5) indicating a type of uncompetitive inhibition [22, 23]. In case of competitive inhibition V_{max}, and in case of noncompetitive inhibitions K_m

remains same. In the context of uncompetitive inhibitions inhibitor binds with the enzyme-substrate complex in an allosteric site other than active site [22, 23].
This study indicates that the isolated terpenoid is a potent inhibitor of AChE of *D. melanogaster* also.

Supportive study in favor of uncompetitive inhibition from bio-informatics approaches

With the help of bio-informatics approaches the supportive study has been done on the crystal structure of AChE of *Torpedo* sp. retrieved from protein data bank (PDB ID: 2C4H). Results obtained from the docking and binding energy calculations reveal that the isolated tetracyclic triterpenoid molecule can bind with the enzyme-substrate complex of acetylcholinestarase. Computational docking studies have shown that the studied terpenoid molecule can binds at peripheral anionic site (PAS), known as inhibitor binding site [18, 19] of acetylcholinesterase-substrate complex with lowest binding energy -79.9526 kcal/mol (Fig. 6). Analysis of intermolecular interactions of generated enzyme-substrate-inhibitor docked structure have identified important key residues *viz.* Tyr70, Asp72, Gln74, Tyr121, Trp279, Ser286, Ile287, Phe288, Phe290, Phe330, Phe331, Tyr334, Gly335 of acetylcholinesterase involving in Vander-Waals interactions with the inhibitor (Fig. 7).

Probable impact of the host plant tetracyclic triterpenoid molecule on the degenerative growth of female Indian lac insect *K. lacca*.

Unlike other homopterans, *K. lacca* can initially disperse in nymphal stages within a few hours just after hatching and becomes sedentary for its entire life cycle, especially the female morph. [12-14]. It also loses all types of locomotory and sensory organs like legs, antenna *etc*. during the course of post embryonic development (Fig. 8; 9) [14]. For locomotion and all type of sensory activities, acetylcholinesterase is the principal enzyme in nerve impulse propagation in living animals from lower Invertebrates to higher Chordates [19, 20]. Several plant secondary metabolites like terpenoids have been known as a potent inhibitor of enzyme AChE [9]. The various primary and tertiary alignments show that AChEs are very evolutionarily conserved enzymes [21]. Probable impact of studied tetracyclic triterpenoid molecule on the degenerative growth after inhibition of AChE is also corroborated by the presence of reduced type of nervous system in female of *K. lacca*. Previous study also shows that Indian lac insect possesses a most degenerative pattern of nervous system in adult female morph, instead of an elaborative nervous system as is evident in nymphal stage[24].

Conclusions

The ingested terpenoid ($C_{30}H_{52}O$) molecule from the host plant by the female *K. lacca*, probably plays a key role by inhibiting the acetylcholinesterase undergoing the process of uncompetitive inhibition which is also supported by bio-informatics studies. This kind of inhibitions of AChE may be one of the responsible factors for the degenerative growth pattern of female *K. lacca* consequently by losing all locomotory organs (legs) and antenna, *etc*. This is most probably caused by the rapid deacceleration of the molar hydrolysis rate of substrate acetylcholine (specific activity) by the enzyme AChE undergoing uncompetitive inhibition by the host plant terpenoid.

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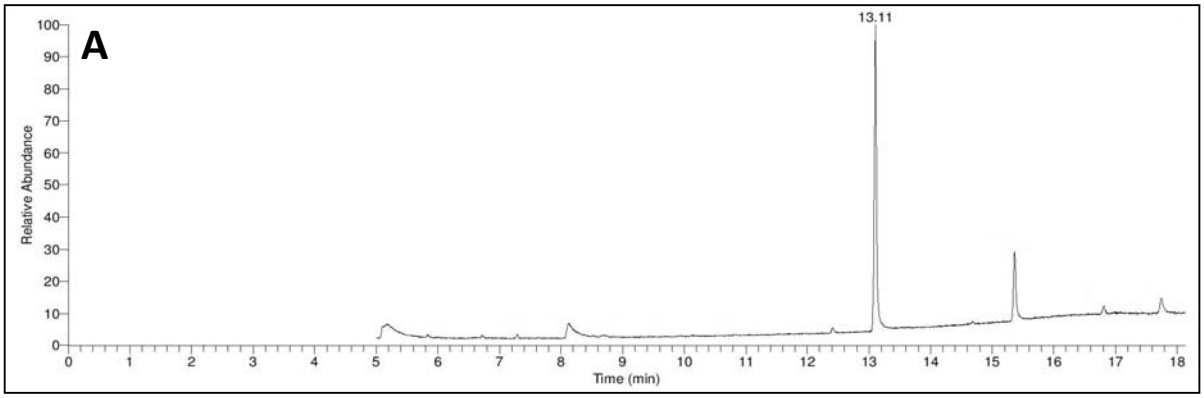
Table 1: Summary of the results on inhibition studies. [↓Refers to decrease; ↑ refers to increase]

Parameters of enzyme kinetics	Absence of Inhibitor	Presence of Inhibitor	*P* Values of Statistical test	Indication
V_{max}	0.140	0.127	0.031	↓
K_m	0.267	0.286	0.022	↑



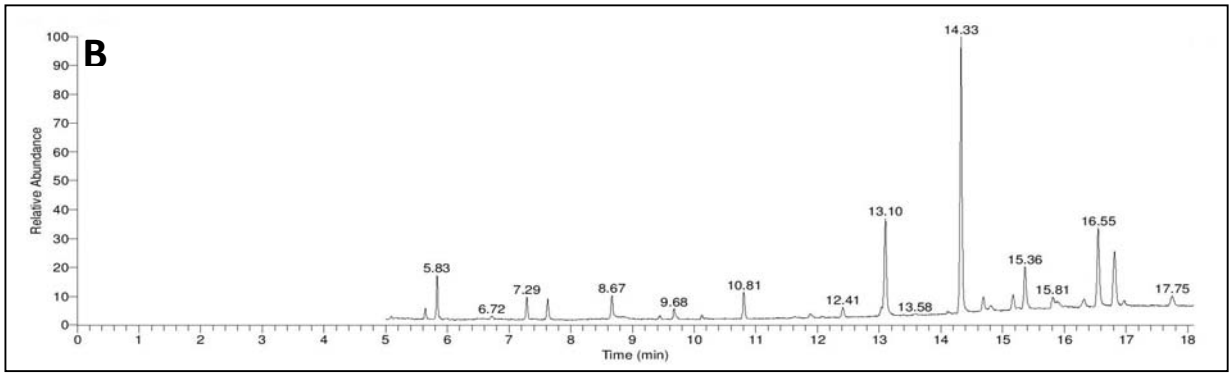


Fig 1: A. Chromatogram of GC of purified tetracyclic triterpenoid inhibitor of AChE present in the bark of host plant *B. monosperma* (Retention time 13.11min). **B.** Chromatogram of GC of semi purified terpenoid moiety present in the bark of host plant *B. momnosperma* complexed with inhibitor molecule (tetracyclic triterpenoid) of AChE.

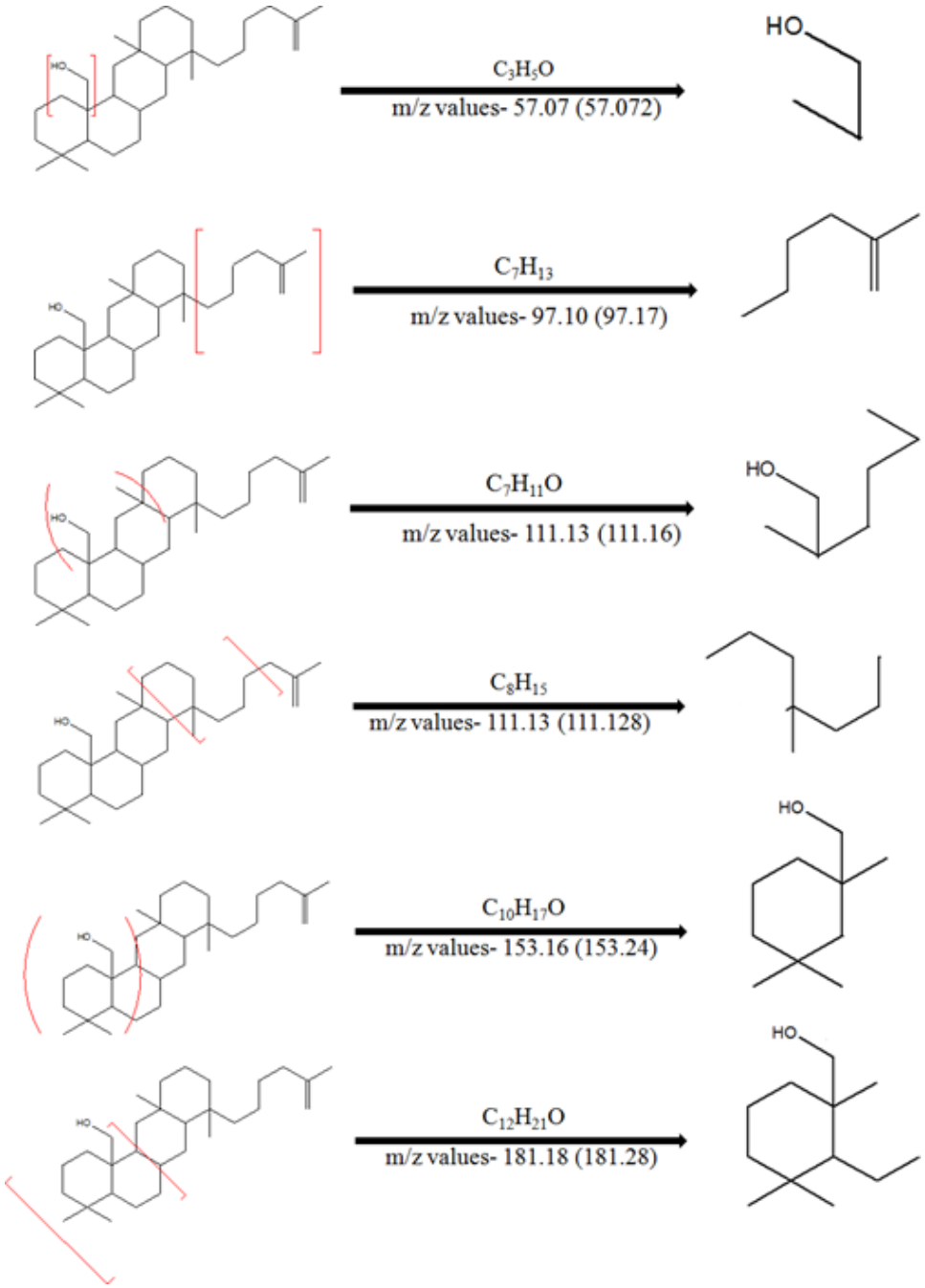


Fig 2: Analysis of the structure based on m/z values of Mass spectrum. Calculated values of mass corresponding to observed values has represented inside the bracket.

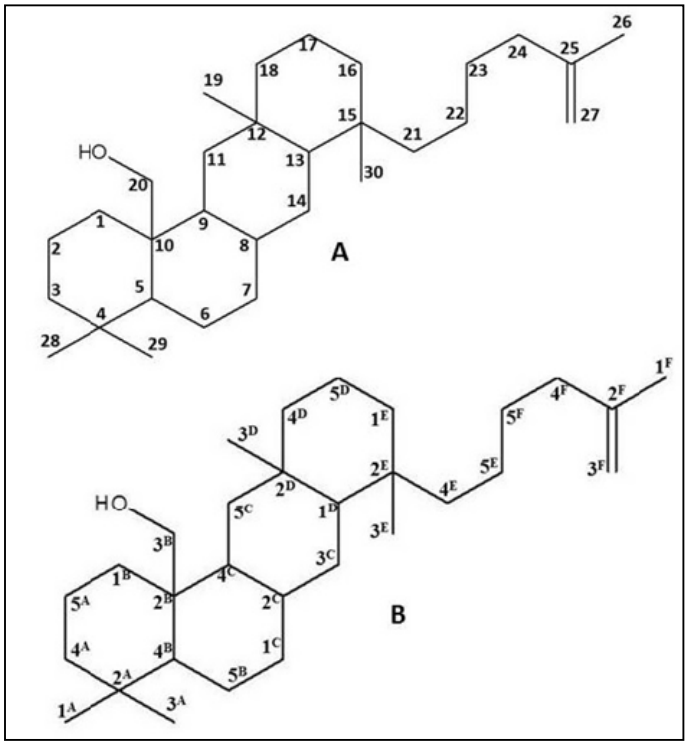


Fig 3: A. Predicted chemical structure of tetracyclic triterpenoid, the AChE inhibitor molecule detected at Rt 13.11 of GC analysis. With proper labeling of atom as the IUPAC rules. B. Orientation of six isoprene units in proper order. [A, B, C, D, E, F each individually represents the six different isoprene units and numerical digits, 1, 2, 3, 4, 5 each represents the five different carbon atom in a single isoprene unit].

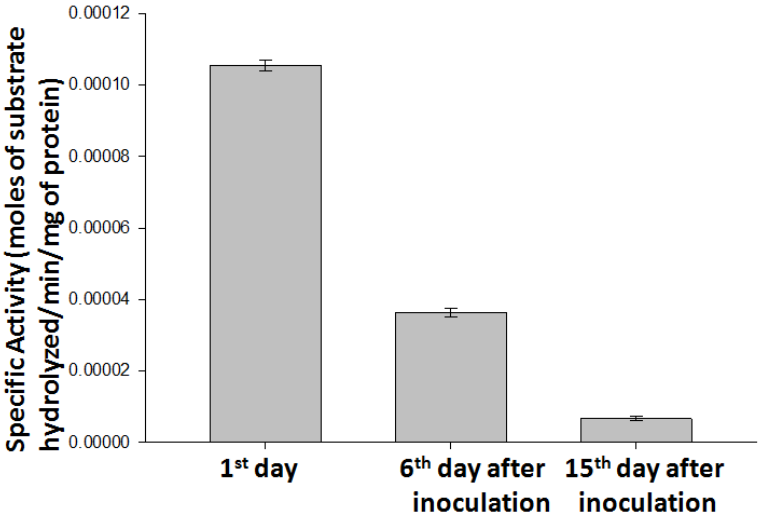


Fig 4: Specific activity (moles of substrate hydrolyzed/min/mg of protein) of AChE at different stages of development of Indian lac insect, *K. lacca* (n=3).

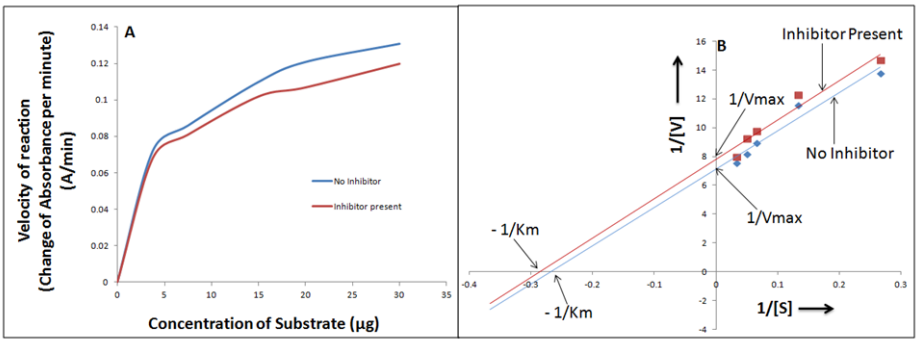


Fig 5: A. MM plot and B. LB plot of AChE kinetics in the presence of studied terpenoid inhibitor as an uncompetitive inhibition (n=3).

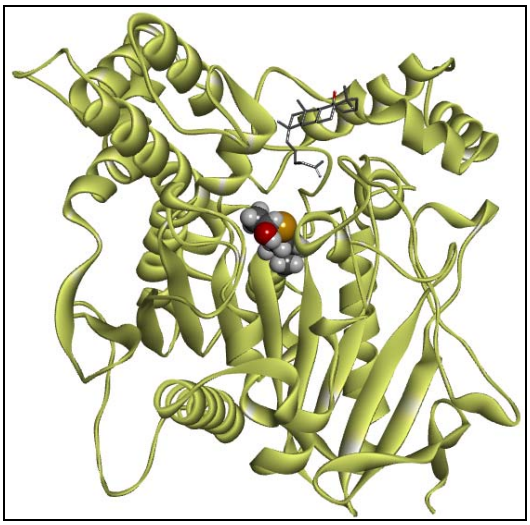


Fig 6: E-S-I Docked complex of acetylcholinesterase (E) with acetylthiocholine (S) (CPK) and Inhibitor molecule (I) (stick) respectively.

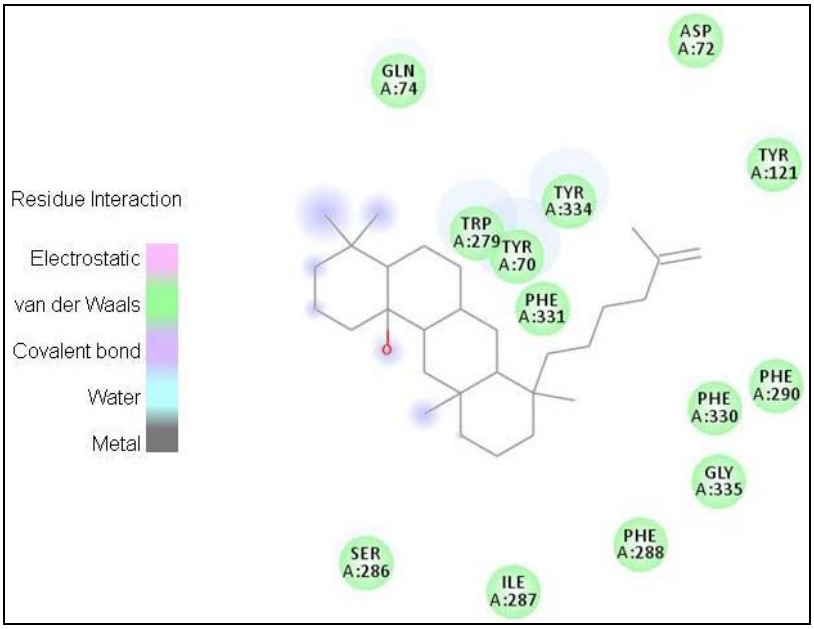


Fig 7: Two Dimensional diagram of receptor-ligand interactions. The solvent accessible surface of an atom is represented by a blue hallow around the atom.

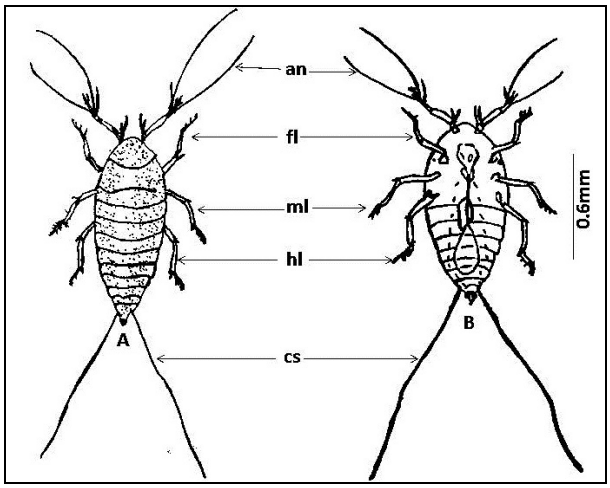


Fig 8: Nymph emerged (FIRST INSTAR) from adult gravid female of *K. lacca* (0.6mm head to last abdominal segment). A. dorsal view, B. ventral view. Abbreviation used: an (antennae), cs (caudal setae) fl (fore lag), hl (hind leg) and ml (middle leg).

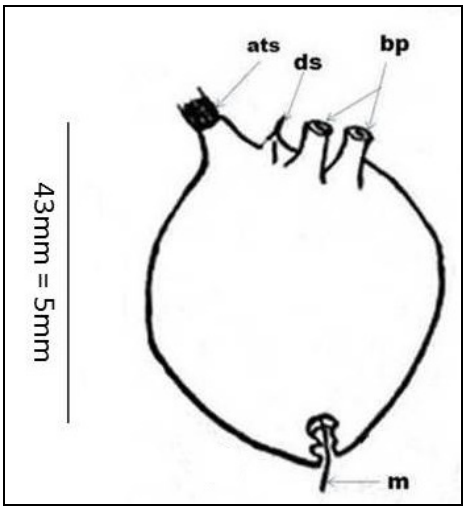


Fig 9: Adult female of *Kerria lacca* out of lac shell (5mm, branchial pore to mouth). Abbreviations used: ats (anal tubercle with setae); bp (branchial pore); ds (dorsal spine); m (mouth).

References

1. Raven JA. Phytophagus of xylem and phloem: a comparison of animal and plant sap feerders. Advance in Ecological Research. 1984; 13:135-234.
2. Ehrlich PR, Raven PH. Butterfly and plants: A study in coevolution. Evoution. 1964; 18: 586-608.
3. Muller KO, Borger. Experimentelle Untersuchungen uder die Phytopthora- resistenz der Kartoffel. Arb.Biol Anst Reichsanst. 1940; 23:189-231.
4. Despress L, David JP, Gallet C. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology and Evolution. 2007; 22(6):298-307.
5. Musser RO, Hum-Musser SM, Eichenseen H, Peiffer M, Ervin G, Murphy JB *et al*. Herbivory: Caterpillar saliva beats plant defences - a new weapon emerges in the evolutionary arms race between plants and herbivores. Nature. 2002; 416:599-600.
6. Zagrobelny M, Rasmussen AV, Jorgensen B, Naumann CM, Lindberg Moller B. Cyanogenic glucosides and plant–insect interactions. Phytochemistry. 2004; 65:293-306.
7. Glendinning JI. How do herbivorous insects cope with noxious secondary plant compounds in their diet? Entomol. Exp. App. 2002; 104:15-25.
8. Feyereisen R. Insect cytochrome P450. In Comprehensive Molecular Insect Science (Gilbert, L.I. *et al*., eds). Elsevier, Amsterdam. 2005, 1-77.
9. Bede JC, Musser RO, Felton GW, Korth KL. Caterpillar herbivory and salivary enzymes decrease transcript levels of Medicago truncatula genes encoding early enzymes in terpenoid biosynthesis. Plant Molecular Biology. 2006; 60:519-531.
10. Fogleman JC. Response of Drosophila melanogaster to selection for P450-mediated resistance to isoquinoline alkaloids. Chem. Biol. Interact. 2000; 125:93-105.
11. Wen Z, Rupasingha S, Gudong N, Berenbaum RN, Schuler AM. CYP6B1 and CYP6B3 of the black swallow tail (Papilio polyxenes): Adaptive Evolution through Subfunctionalization. Molecular Biology and Evolution. 2006; 23(12):2434-2443.
12. Pushker AK, Kaushik S, Lakhanpaul S, Sharma KK, Ramani R. Preliminary Phytochemical Investigation on the Bark of Some of the Important Host Plants of Kerria lacca-The Indian Lac Insect. Botany Research International. 2011; 4:48-51.
13. Kaushik S, Pushkar AK, Lakhanpaul S, Sharma KK, Ramani R. Investigations on some important host plants of Kerria lacca with reference to phloem distance. Eur Asian Journal of Bio Sciences. 2012; 6:32-38.
14. Ghorai N. Lac-Culture in India. International Books and Periodicals Supply Services, New Delhi. 1995, 6-117.
15. Chakraborty S, Dutta T, Rai K, Ghosh J, Saha SK, Biswas S *et al*. Preliminary investigation on terpenoids present in the bark of major host plants, and lac-encrustations made by the Indian lac insect Kerria lacca (Kerr.) with respect to host plant selections. Journal of Entomology and Zoology Studies. 2016; 4(4):591-594.
16. Sharma KK, Jaiswal AK, Kumar KK. Role of lac culture in biodiversity conservation: issue at stake and conservation strategy. Current Science. 2006; 91:894-898.
17. Ellman GL, Courtney KD, Andres V, Feather-Stone RM. Anew and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88-95.
18. Johnson G, Moore SW. The peripheral anionic site of acetylcholinesterase: structure, functions, and potential role in rational drug design. Curr. Pharm. Des. 2006; 12(2):217-215.
19. Massoulie J, Anselmet A, Bon S, Krejci E, Legay C, Morel N *et al.* The polymorphism of acetylcholinesterase: Post–translational processing, quaternary associations and localization. Chemico–Biological Interactions. 1999; 42(29):119-120.
20. Massoulie J. The origin of molecular diversity and functional anchoring of cholinesterases. Neurosignals. 2002; 11:130-143.
21. Wienser J, Kriz Z, Kuca K, Jun D, Koca J. Acetylcholinesterases – the structural similarities and differences. J. Enzyme inhib Med Chem. 2007; 22(4):417-424.
22. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. W.H Freeman and Company, New York. 2008, 202-213.
23. Berg JM, Tymoczko JL, Stryer L, Biochemistry WH. Freeman and Company, New York. 2002, 329-349.
24. Negi PS. The Alimentary Canal, its Appendages, Salivary Glands and the Nervous System of the adult Female Lac Insect, Laccifer lacca, Kerr (Coccidae). Bulletin of Entomological Research. 1934; 25(4):541-550.