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Insilico and Proteomic Analysis of Dengue Vector Midgut Proteins Treated by *Aegle marmelos* Bioactive Compounds

Sugapriya Menaga Paulraj, Maheswaran Baskaran, Sakkanan Ilango, Geetha Paramasivam and Joseph Sebastinraj

Abstract: Dengue disease is a mosquito vector borne viral disease which is transmitted mostly by the *Aedes aegypti* mosquito vector species. Other viral diseases such as chikungunya, yellow fever and zika are also caused by this vector. After feeding on a DENV-infected person, the virus replicates in the mosquito midgut then it distributes to other tissues. Medicinal plant species contains wide series of phytochemicals such as primary and secondary metabolites that produce biological activities and defenses against mosquitoes. In the current study, a methanol leaf extract of *Aegle marmelos* was assessed for larvicidal efficacy and midgut alteration of third instar *Ae. aegypti* larvae. After the larvicidal bioassay, the probit analysis identified Lc50 in this extract that killed the larvae at the concentration of 49 ppm. Proteomic analysis and *in-silico* studies revealed that the predicted protein could disrupt the larvae midgut because of the *Aegle marmelos* natural bioactive compounds and secondary metabolites. Our approach is to identify the inhibitor proteins that bound to midgut of *Ae. aegypti* larvae after treatment with the *Aegle marmelos* bioactive compounds by using computational proteomic analysis.

Index Terms - Dengue, *Aedes* mosquito, MALDI, midgut & proteome.

I. Introduction

Dengue, Dengue Shock Syndrome and Dengue Hemorrhagic Fever (DHF) are an important vector borne viral diseases. WHO said the prevalence of dengue has grown-up dramatically worldwide in these decades and the largest number of dengue cases ever reported was in 2019.

There are 2.5 billion people Worldwide are at risk of dengue fever (Guzman *et al.,* 2010). In India, a sum of 136,422 cases and 132 deaths were recorded in 2019 which is the maximum number of cases when compared to previous year. Dengue virus contains, four serotypes are DENV1, DENV2, DENV3 and DENV4 and also several subtypes. One serotype can produce lifelong immunity but it is merely a partial immunity besides the other serotypes of reinfection. All these serotypes have interaction with the host and displayed unique features based upon its response (Ekta and Neha, 2014). The structural features of these virus comprises of three structural proteins such as capsid, membrane and envelope and seven (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) non-structural proteins.

World's insecticide market of phytochemicals was reported as one per cent (Ghosh *et al.,* 2012). Phytochemicals may help as these are relatively safe, cheap, environment friendly, biodegradable and readily available in all over the world. Controlling this medically important vector is really a challenge of emerging resistance to chemical pesticides. Medicinal plants contain a wide variety of phytochemicals such as alkaloids, polyines, coumarins, peptides, flavonoids, terpenoids, polyphenolics and saponins that have demonstrated therapeutic effect against a wide range of viruses, their entry and replication (Idrees and Ashfaq, 2013).

The plant *Aegle marmelos* belongs to Rutaceae family, also known as bael, a spiny tree. Native to India, it is an important medicinal herb and widely used in medicinal systems (Atul, 2012). These plants consists various phytochemicals and are responsible for its medicinal value. Hence this study, aims at investigating the larvicidal efficacy of the leaves methanol extract of *Aegle marmelos* on the III instar *Ae. aegypti* larvae. Bradford method was used to estimate the infected gut protein concentrations (Stephenson, 2010). Further the plant extract that exhibits larvicidal activity was used to study proteomic analysis of the larvae midgut and its related proteins were predicted by computational analysis. We have isolated the protein present in

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Sugapriya Menaga Paulraj & Sakkanan Ilango, *[1]Post Graduate and Research Department of Zoology,*
Maheswaran Baskaran & Geetha Paramasivam, *[2]Department of Biotechnology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, India.*
Joseph Sebastinraj, *[3]Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India.*
Email: menagadurai@gmail.com

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mosquito midgut using SDS-PAGE after intoxication of *Ae. aegypti* larvae with plant extract *Aegle marmelos* to discover novel inhibitor proteins. In this contest, proteomics analysis might be very useful for the identification and development of new drug targets to advance the insecticide resistance on midgut larvae.

The proteomic analysis was conducted to find *Ae. aegypti* midgut proteins after intoxicated with the plant extract with the help of Mass spectrometry (Whiten *et al.,* 2018). Our approach combined with SDS PAGE and MALDI to identify the DENV inhibitor proteins that interacted with the *Ae. aegypti* midgut.

II. Material And Methods

1) Plant Material Collection and Extraction

The studied plant leaves of *Aegle marmelos* was freshly collected from Sivakasi, Viudhunagar District in the state of Tamil Nadu, India. The studied plant was identified with voucher specimen. The leaf samples were washed and rinsed thrice with sterile water to remove the contaminants and then shade dried at room temperature to remove the moisture. Then, the dried samples were made into fine powder. The fine powders were extracted twice with 95 % methanol solvent in Soxhlet apparatus, to obtain the phytochemicals. The extract was then concentrated under reduced pressure in the rotary vacuum evaporator until the solvents evaporated completely at 45^0C to get semisolid mass of crude extracts and then freeze dried at -80^0C to obtain solid residue (George, 2008).

2) Larvae Collection & Larvicidal bioassay

The mosquito larvae *(Aedes aegypti*) were collected from the Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu and India. Mosquito culture was maintained at the temperature of 28 to 29°C, 80 to 85% relative humidity under the light: dark photoperiod cycle of 14:10 h. The larvae were reared in plastic tray containing tap water and fed brewer's yeast and dog biscuits powder in the ratio of 1:2. The water has been changed on each alternate day.

According to the guidelines of World Health Organizationm, the larvicidal bioassay was performed on third instar larvae (WHO, 2005). To 150ml of de-chlorinated tap water taken in a beaker appropriate volume of 1% stock solution of *Aegle marmelos* methanol extract fractions were added and mixed to obtain different concentrations. Third instar larvae of *Ae.aegypti* in 25 numbers were released to each concentration and provided with larval feed and test was conducted in five replicates. There are two controls were maintained (one with 150ml water alone and the other with 150ml of water containing maximum volume of acetone in the test sample). Primary larvicidal screening was carried out with 100, 500 & 1000 ppm concentrations to identify the active range for the further bioassay with the extract. Afterwards of the preliminary analysis, a test range of 50,100, 150, 200 and 250 ppm were fixed to identify the Lc50 and Lc90 values and outright of per cent mortality recorded after 24 and 48hrs of exposure.

3) Statistical Data analysis

Considering the percentage mortality of the larvae after 24 and 48hrs in different concentrations, Lc50 of the test fractions, we calculated using probit analysis and IBM SPSS Statistics 23 software. Per cent mortality was calculated based on the Abbott's formula (Abbott, 2010) and the statistical analysis was carried out based on the log-dose response (Finney, 1971). The significant difference in Lc50, Lc90, and 95% Fiducial limits and also the slope values are calculated.

4) Preparation of midgut protein extracts

In this study, the proteomic study on the third instar larvae of *Aedes aegypti* mosquito vector was carried out after intoxication with various concentration of *Aegle marmelos* methanol extract for 24 and 48hr period. After 48 hr the dead larvae from the treatment were collected. For the midgut sample preparation of SDS, larvae midguts were collected under a microscope by dissection using ER Buffer consist of protease inhibitor in the conc. of 1 μL/mL (Sigma P9599) (English and Reddy, 1989). The midgut was completely separated from other mosquito parts by standard procedure (Butler and Deana, 2014). Centrifugation of midgut sample was done at 12,000 rpm for 15 mins at ice cold condition. In to new eppendorf tubes the supernatants were collected and stored in -80°C till further electrophoretic analysis. The photographs of both control and tested larvae midgut was captured in the light microscope attached with a digital camera. Protein concentration in the midgut was estimated by Bradford assay which was compared with standard BSA protein (Bradford, 1976).

5) Protein profiling

SDS-PAGE analysis of both the control and treated *Aedes* midgut larvae extract was performed by using the standard protein isolation (Laemmli, 1970). For sample preparation, 50 mg of protein extract was mixed with sample loading buffer, kept in water bath at 60 to 65°C for 2min and electrophoresed on 15% of separating gel and 4% stacking gel mix (Pandiarajan, *et al.,* 2011). The SDS – PAGE gel was stained with Coomassie Brilliant Blue R-250 for six hour. Washed the gel twice

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with the double distilled water and kept in a destaining solution for the appearance of bands in the gel.

6) In-gel protein digestion

The identified band in SDS-PAGE gel was excised; destaining was carried out with methanol and incubated with 200mM ammonium bicarbonate. Dehydration of the gel was done with acetonitrile solution, dried under vacuum followed by rehydration and tryptic digestion.

7) Protein Identification by (MALDI-TOF) & Bioinformatics analyses

In-gel Trypsin digestion and Mass spectrometry analysis was performed in the Molecular Biophysics Unit, IISC, Bangalore, India. The m/z ratio peaks obtained from the MALDI MS analysis was subjected to online MASCOT search software tool (Matrix Science Inc., Boston, U.S.A.) to obtain the peptide sequences. The search parameters were set as following, the fixed modification is set to carbamidomethyl C (Cysteine) and variable modification is set to oxidized M (Methionine), missed trypsin cleavage site is set to 1.

In the mascot search engine, predicted protein should significant with a p-value less than 0.05. Identified sequences were searched against with Drosophila organisms in Swissprot database sequence for the functions detection. Vectorbase database and BLAST server was used to validate the protein and the protein interactions were predicted by using STRING database.

III. Results And Discussion

The primary phytochemical screening of the methanolic extract of the *Aegle marmelos* leaves showed the presence of various primary and secondary metabolites in the extract such as protein, phenols, tannins, steroids and titerpene compounds. The current study indicates that the most active ingredient isolated can be act as a best larvicidal agent against the third instar larvae of *Aedes aegypti* and also showed development distortion. This insect feed on these secondary metabolites encountering toxic substances with non-specific effects on molecular targets and in turn affects physiology in many different ways at different receptor sites (Ghosh *et al.,* 2012).. High level mortality was noticed in the *Aegle marmelos* and which may be due to the phytochemicals in the extracts which arrests the metabolic activities of larvae. The larvicidal efficacy of *A. marmelos* against the *Aedes aegypti* third instar larvae was fixed at 50, 100, 150, 200 and 250 ppm. The highest toxicity in the bioactive compounds observed at 24 hours Lc50 values are 59 and 49 and at 48 hours Lc90 values are 114ppm and 108 ppm respectively (Table 1).

Table 1: Larvicidal Toxicity of *Aegle marmelos* methanol leaf Extract The Dengue Vector, *Aedes aegypti*

	Per cent Mortality (ppm)					Lc50	95%	Lc90	95%	Slope ±SE	χ^2 (df=3)	Reg. equation
	50	100	150	200	250	(ppm)	(LCL-UCL)	(ppm)	(LCL-UCL)			
24hr	08	32	60	88	96	59	50.56 - 68.11	114	95.95-150.97	4.504±.682	2.23*	y = 0.928x - 12.8
48hr	20	44	68	88	100	49	39.68 - 57.39	108	88.11-150.59	3.711±.592	3.82*	y = 0.816x + 2.8

*Control-Nil mortality, UCL & LCL - Upper & Lower confidence Limit, X^2 - Chi-square value, df - degrees of freedom, *Significant at P < 0.05 level.*

No significant mortality for control assays. The methanol leaf extract of *Aegle marmelos* treated larvae revealed the damage disruption around the midgut The midgut region was completely disrupted with shrunken bodies. Thus this extract obviously led to disruptions in growth of *Ae. aegypti* third instar larvae. *A. pinnata* might have contributed in body effects of larvae and their study indicated that the potential application of this plant phytochemicals as mosquito larvicidal agent (Zulkrnin *et al.,* 2018). The *S. terebinthifolius* plant derived bioactive compounds have been reported for larvicidal effects in the mosquito midgut (Procópio *et al.*, 2015), similar to the *Aegle marmelos* leaf extract. The damage to midgut cells of *Ae. aegypti* larvae caused by the *Aegle marmelos* methanol leaf extract may have digestive dysfunction in the larval midgut, and distortion in the growth of larval development.

Primarily, the *A. marmelos* treated larvae midgut protein sample was quantified using Bradford method BSA as standard (Table 2) and the quantification of protein was plotted in the Fig.1. *Ae. aegypti* midgut protein of larvae was analyzed quantitatively by using Colorimeter and the OD value was measured at 595nm. The present investigation showed that the protein concentration was found to vary in the treated and untreated *Ae. aegypti* midgut proteins. The mean protein concentration was effectively recorded for both untreated larvae (1237.2µg/mL) and *Aegle marmelos* leaf extract treated larvae (1078.6µg/mL). In the present study, total protein content was significantly higher in untreated larvae than the *Aegle marmelos* leaf extract treated. The results showed that concentration of protein seen higher in untreated larvae reflects a high rate of protein synthesis in the control. Thus, the present findings suggest that there is a disruption taking within the midgut protein

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synthesis. Ratten (2010) reviewed the mode of action of secondary metabolites on insect body and documented several physiological disruptions.

Table 2 Quantification of *Aegle marmelos* treated *Ae. aegypti* midgut protein by Bradford's method

Test tube	Conc. µg/ml	BSA (µl)	Distilled H_2O (µl)		OD (595 nm)
S1	10	100	1900	Bradford Reagent 3 ml	0.035
S2	20	200	1800		0.662
S3	30	300	1700		0.695
S4	40	400	1600		1.466
S5	50	500	1500		1.864
T1	1237.2	100	1900		1.116
T2	1078.6	100	1900		0.978

S1 to, S5 – Standard protein BSA; T1– Untreated; T2– Treated with Aegle marmelos; Control: 5ml Bradford's reagent;

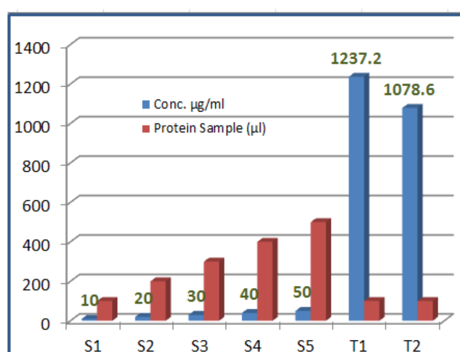

Fig. 1 *Aegle marmelos* treated *Ae. aegypti* third instar larvae midgut protein quantified by Bradford's method

In this study, the proteomic analysis of *Aedes aegypti* third instar larvae midgut was evaluated after treated with various concentrations of *Aegle marmelos* leaf extract that kills the larvae at 48hrs of incubation. To know the peptide responsible for the larvae structure modification, we decided to separate the midgut protein extract in 15% SDS-PAGE. The size of the band obtained in the gel was between 20 to 66 kDa in range. This result is corroborated with (Abbas *et al.*, 2013), they reported that six protein bands were identified ranged from 16.6 to 75 kDa in molecular weight.

Previous studies of midgut epithelial cell structure and function have revealed some information on the cell types defined in *D. melanogaster* are also established in *Ae. aegypti* (Fernandes *et al.,* 2014). The pathway induced in midgut of DENV2-primered mosquitoes were identified as Notch transcription (Serrato *et al.,* 2018) and it is an important defensive mechanism against dengue virus infection.The 57 kDa protein possesses dengue viral binding protein property and it has proven by previous studies in the *Aedes* midgut. The DS3 strains of *Ae. aegypti* total purified protein resolved in SDS showed that they were in the range of 57 and 67 kDa (Muñoz *et al.,* 2013). Based on this, we also focused on the ~57 kDa fragment was eluted from the SDS-PAGE gel (Fig. 2) exposed to MALDI-MS analysis followed by tryptic digestion and the chromatogram results displayed in the Fig. 3.

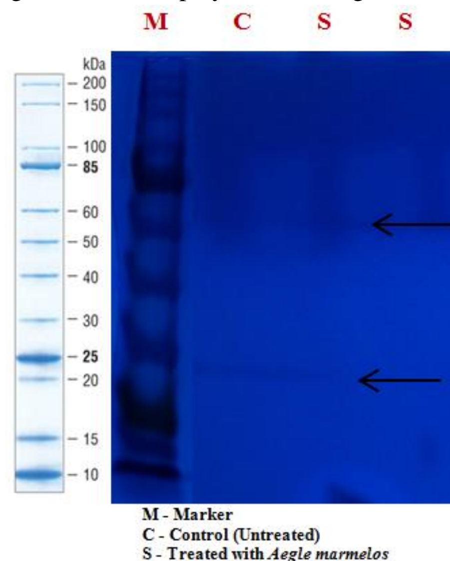

Fig. 2: Protein profile of *Aegle marmelos* methanol leaf extracts treated and untreated *Aedes aegypti* Midgut.

Mosquito midgut proteins were separated by SDS-PAGE with CBB stained gel. On the right side, shows molecular weights of the proteins and the left shows marker protein.

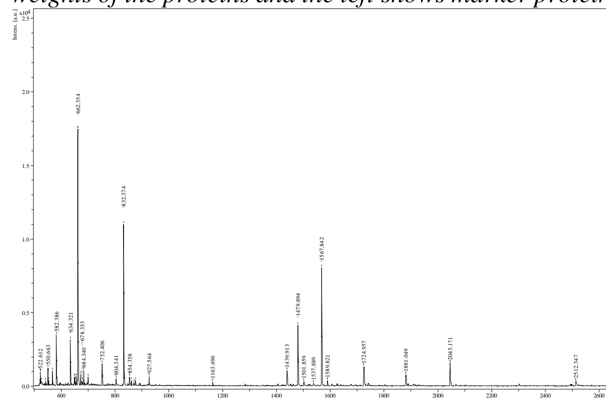

Fig. 3 MALDI MS tryptic digested peptide of *Aedes aeygpti* midgut protein treated by *Aegle marmelos* plant extract and the peptide masses were used for protein database searches

The peptide masses list were searched against the protein sequence database, and predicted protein as the product of gene Cyp313a1 of cytochrome P450 sequence of *Drosophila melanogaster* which is a 56 kDa protein (Fig.4). The experimentally determined seventeen masses cover only 17 per cent of the protein sequence. Table 3 shows the observed and calculated masses of peptide and their sequence assignments. Among the 17 peptide masses identified, 8 peptides covered in the predicted protein *Drosophila* sequence.

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The obtained sequence was compared with the non-redundant protein sequence database specified with the *Aedes aegypti* organism using BLASTP software.

The results confirmed that the predicted protein belongs to *Aedes aegypti* cytochrome P450 sequence with 42 per cent identity Fig. 5.1 to 5.3. Further, the longest matched peptide as query sequence in fasta format (NCIGSKYAMMSSKFALCR) was subjected to a Vectorbase BLASTP tool for similar sequence identification from *Aedes* species sequences stored. The analysis was based on Vectorbase database *Ae. aegypti* sequences and related similarity. Interestingly the query is also perfectly aligned with the eight cytochrome P450 peptides of *Aedes aegypti* sequences with more than 55 per cent identity and score. Among these, four peptide sequences AAEL012772-PA, AAEL017136-PB, AAEL012766-PA and AAEL003748-PA showed biologically significant result once viewed their E-values (Table 4). By mascot searches, Muñoz *et al.* (2013) identified the 57 – 67 kDa proteins were enolase, beta-ARK, translation elongation factor EF-1 alpha/ Tu and cadherin. Peptide Mass Fingerprint data analysis couldn't show the same mass of different peptides (Sechi and Chait, 1998; He *et al.*, 2008).

But the protein identified by this current study does not match with any other *Aedes aegypti* midgut proteins studied so far this means that the *Aegle marmelos* plant leaf extract might altered or influence the protein present in the midgut membrane. The total mass chromatogram *of Aedes aegypti* third instar larvae treated with *Aegle marmelos* plant leaf extract midgut proteins obtained, after that MALDI/MS spectrum of mascot search identified four upregulated proteins with *Drosophila melanogaster* are AT-rich binding protein, Bomanin, Accessory gland-specific peptide and Eukaryotic translation initiation factor at peaks 662.354, 832.374, 1479.894 & 1567.842 m/z respectively (Table 5). Structural and functional networks of protein-protein interactions of identified proteins were analyzed and predicted using STRING 11 analysis software. STRING analysis can be used to understand the cellular machinery at the system level and this information can be implicated in modeling, annotation and pathway studies (Fig. 6).

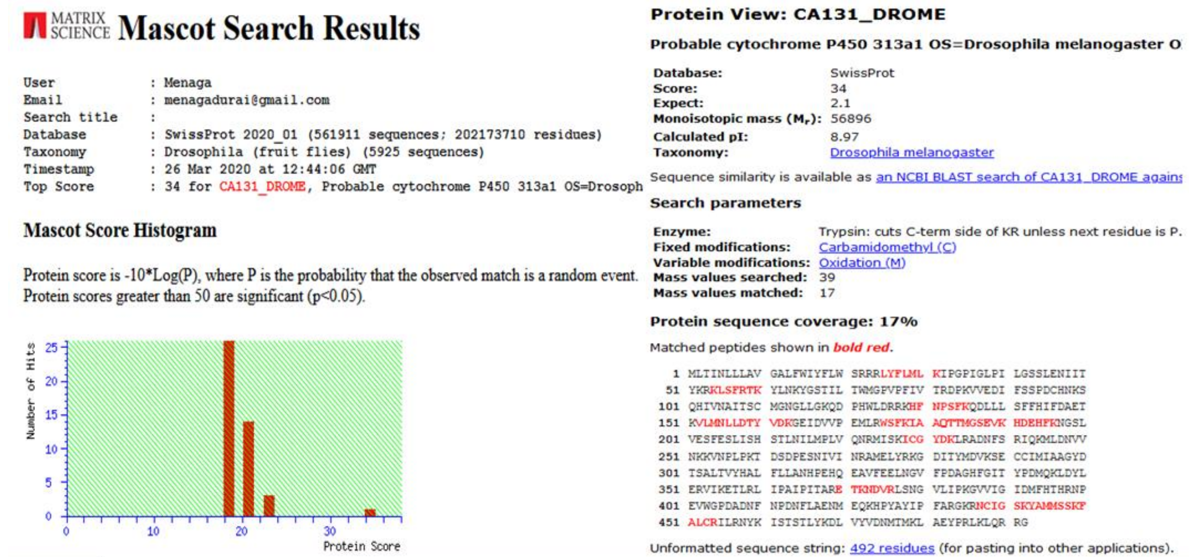

Fig. 4 MALDI peptide masses analyzed by Mascot server against Swissprot database shows that the predicted protein has 17% sequence coverage with *Drosophila* Cytochrome P450; Matched peptide sequences shown in bold red

Input: BLAST Query Sequence in Fasta format retrieved from Swissprot Database

>sp|Q9VFJ0|CA131_DROME Probable cytochrome P450 OS=*Drosophila melanogaster* GN=Cyp313a1
MLTINLLLAVGALFWIYFLWSRRRLYFLMLKIPGPIGLPILGSSLENIITYKRKLSFRTKYLNKYGSTILTWMGPVPFIVTRDPKVVEDIFSSPD
CHNKSQHIVNAITSCMGNGLLGKQDPHWLDRRKHFNPSFKQDLLLSFFHIFDAETKVLMNLLDTYVDKGEIDVVPEMLRWSFKIAAQTT
MGSEVKHDEHFKNGSLVESFESLISHSTLNILMPLVQNRMISKICGYDKLRADNFSRIQKMLDNVVNKKVNPLPKTDSDPESNIVINRAM
ELYRKGDITYMDVKSECCIMIAAGYDTSALTVYHALFLLANHPEHQEAVFEELNGVFPDAGHFGITYPDMQKLDYLERVIKETLRLIPAIPIT
ARETKNDVRLSNGVLIPKGVVIGIDMFHTHRNPEVWGPDADNFNPDNFLAENMEQKHPYAYIPFARGKRNCIGSKYAMMSSKFALCRI
LRNYKISTSTLYKDLVYVDNMTMKLAEYPRLKLQRRG

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Table 3: Molecular Masses for Tryptic Peptides Identified from MALDI Search against Mascot server for the *Aedes aegypti* Midgut protein Treated with *Aegle marmelos* plant extract

Mass (Da[b])			Seq. position			
Observed	Expected	Calculated	Start	End	M	Peptide Sequence[a]
522.612	521.6047	521.2962	55	58	0	K.LSFR.T
568.172	567.1647	566.2853	175	178	0	R.WSFK.I;K.IAAQTTM[c]GSEVKHDEHFK.N
650.079	649.0717	649.3911	54	58	1	R.KLSFR.T
651.298	650.2907	649.3911	54	58	1	R.KLSFR.T
666.075	665.0677	665.3319	450	454	0	K.FALCR.I
678.353	677.3457	677.3166	437	442	0	R.NCIGSK.Y
752.406	751.3987	750.4388	55	60	1	K.LSFRTK.Y
754.396	753.3887	754.332	228	233	0	K.ICGYDK.L
832.374	831.3667	832.3459	443	449	0	K.YAMM[c]SSK.F
861.133	860.1257	860.4352	370	376	1	R.ETKNDVR.L
876.344	875.3367	875.429	129	135	0	K.HFNPSFK.Q
927.564	926.5567	926.5299	25	31	0	R.LYFLM[c]LK.I
1439.913	1438.9057	1438.7378	152	163	0	K.VLM[c]NLLDTYVDK.G
1479.894	1478.8867	1479.6672	443	454	1	K.YAMM[c]SSKFALCR.I
2045.171	2044.1637	2043.9684	179	196	1	K.IAAQTTM[c]GSEVKHDEHFK.N

__a__ The peptides cover 17% sequence coverage and 17 peptides were predicted. The peptide mass accuracy (better than ±1.2 Da) and identify the protein in the Swissprot protein sequence database; __b__ Monoisotopic, neutral masses; __c__ Met is oxidized.; __M__ - Missed cleavage

Table 4 : Similarity Search of Cyt P450 mascot predicted protein against the *Aedes aegypti* Sequence database at Vectorbase (*Aegle marmelos* Treated)

Blast Query for Vectorbase :		> Cyt P450 peptide(fasta) NCIGSKYAMMSSKFALCR		
Similar Peptide	**Gene**	**E-value**	**Score**	**Identity**
AAEL012772-PA	CYP325G3	0.023	68	70.60%
AAEL000320-PA	CYP325T1	0.36	59	68.80%
AAEL017136-PB	CYP325V1	0.053	65	66.70%
AAEL012766-PA	CYP325G2	0.096	63	64.70%
AAEL006044-PA	CYP325Q1	0.28	60	62.50%
AAEL003748-PA	CYP9AE1	0.045	66	61.10%
AAEL005775-PA	CYP325R1	0.53	57	56.30%
AAEL007812-PA	CYP4H32	0.46	58	55.60%

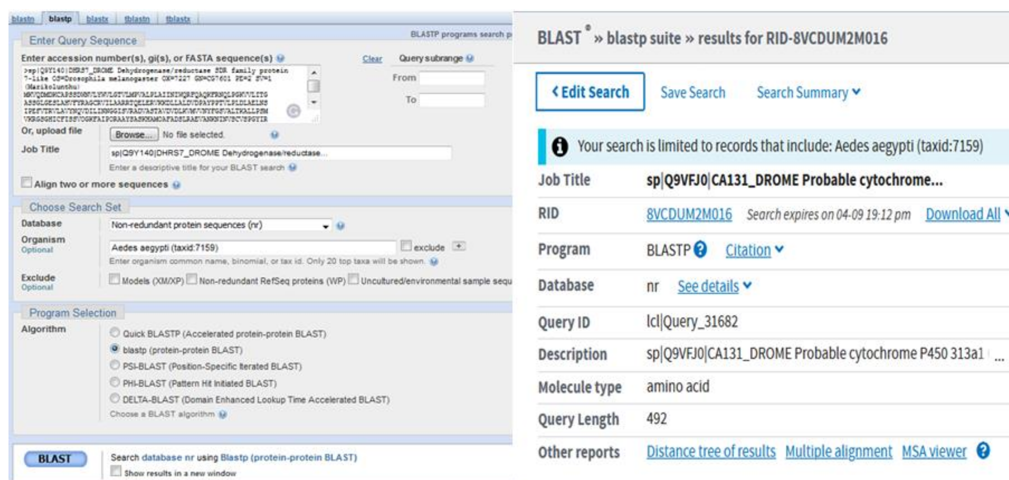

Fig. 5.1 Setting up of BLASTP search of the protein sequence predicted by MALDI Mascot search against the NCBI *Aedes Aegypti* (taxid:7159) organism Non-redundant Protein sequence database

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Fig. 5.2 BLASTP Output: a) Query details; b) A Graphical overview of all the similar sequences;c) List of BLASTP hits that produce significant alignments with our query sequence and contains links to the NCBI databases

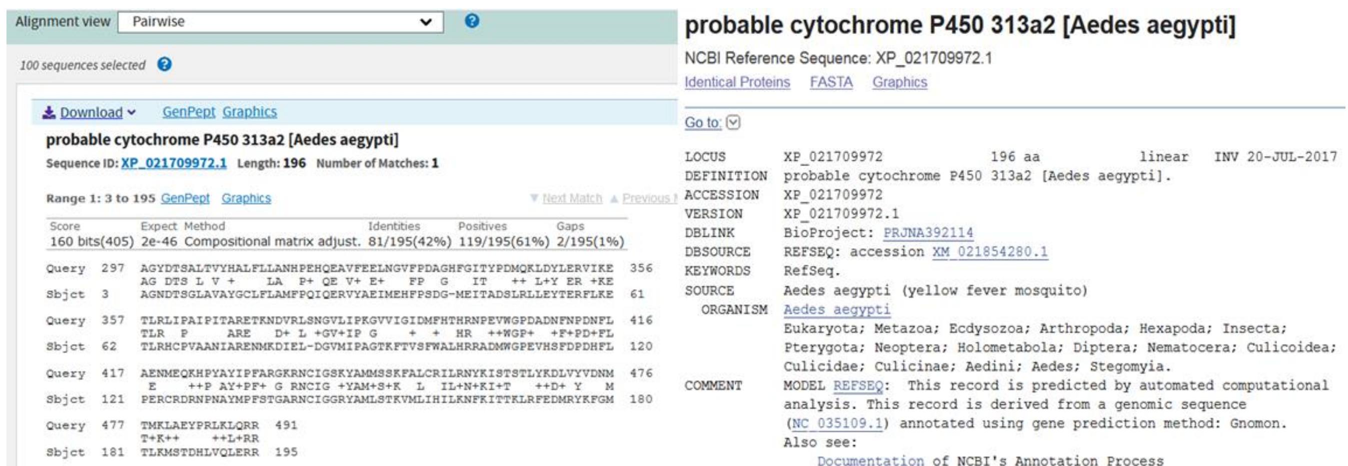

Fig. 5.3 a) BLASTP alignment between the peptide sequence predicted (Query) with the *Aedes aegypti* Cytochrome P450 sequence (Sbjct); b) *Aedes aegypti* Cytochrome P450 retrieved from NCBI Sequence database

Table 5: Peptides identified as up-regulated in midgut of *Aegle marmelos* treated *Ae. aegypti* larvae

Peptide mass (Da)	Peptide sequence	Similar Sequence *Drosophila melanogaster*	Score	Sequence coverage	pI
662.354	FKYKSRMELHRVVHSKER	AT-rich binding protein	57	5%	6.75
832.374	K.VCNIRGD	Bomanin-068 - immune-induced peptide toll signalling	19	17%	7.82
1479.894	R.KPTKFPIPSPNPR.D	Accessory gland-specific peptide	19	23%	10.1
1567.842	K.GNDDDIQDGLVHIR.I	EK Translation initiation factor	17	12%	6.82

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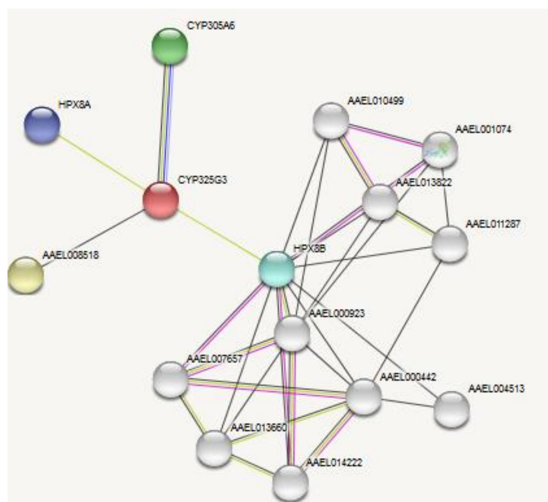

Fig. 6 STRING PPI network of Cytochrome P450 protein identified in the of *Aegle marmelos* Treated *Ae. Aegypti* larvae midgut. ID numbers denote sequence accession numbers in the Swissprot. Interaction networks are shown in the confidence view with color lines.

Green colour depicts neighbourhood; Red colour: Gene fusion; Pink colour:Experiments; Light green colour: Text mining; Blue colour:Cooccurrence; Dark blue colour: Coexpression; Purple colour:Homology; and circle nodes indicated different proteins. Interaction networks are shown in evidence view

The available association network was studied for the protein, i.e. AAEL012772-PA (Cytochrome P450) matched with monooxygenase and Heme peroxidase has homologs with *Aedes, Culex* and *Anopheles* species with the p-value of 0.566. Detailed functional analysis of these proteins was carried out using GO and other bioinformatics algorithms. The biological process of the identified peptide involved in oxidation-reduction process and the molecular function depicts the monooxygenase activity.

The xenobiotics reduction or oxidation process of cytochrome P450 monooxygenase (CYP) gene was studied (Saavedra *et al.,* 2014) and it's represented in *Aedes aegypti* detoxification genes. P450s are involved in drug metabolism (Werck and Feyereisen, 2000) and insecticide resistance. The pyrethroid influence in CYP4 expression may cause adverse effect in *A. albopictus* (Avicor *et al.,* 2014). Ilango *et al.* (2007) reported that C-methylated flavone from C. lanceolatus is responsible for the larvicidal activity. The active compounds identified will play a role in developmental duration of the mosqutoes such as *C. quinquefasciatus* and *A. stephensi* (Ilango *et al.,* 2016).

IV. Conclusion

At present, there is no potent medicine for dengue making the laboratory oriented investigation system is an important need and an essential tool to control the *Aedes aegypti* mosquito vector control and also environmental friendly. Dengue virus replication mainly occurs in mosquito midgut and plays a mail role in the transmission of this disease to humans. Ecofriendly, cost effective control of this mosquito by natural bioactive compound is necessary. In-silico proteomic analysis is essential for developing novel vector control strategies, and to identify dengue viral protein receptors on the midgut which will help in future research. In conclusion, our study revealed that *Aegle marmelos* leaf extract had potencial application as larvicide.

The computational proteomic analysis highlights the potential effect of *Aegle marmelos* leaf extract against the *Ae. aegypti* third instar larvae and exhibited an induction of structural disorganization in the midgut epithelial cells. The identified phytochemicals and their ability to control this vector species is either by their insecticidal property or by growth disruption. These phytoextracts could be purified further and used as biological insecticides instead of synthetic chemicals, which currently is the major means of mosquito vector control and also environmental friendly. Further studies will be indispensable to validate the pharmacological properties and make potent drug at cheap cost from of natural phytochemicals.

Conflict Of Interest

The authors declare that they have no conflict of interests.

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AUTHORS PROFILE

Sugapriya Menaga Paulraj, M.Sc. M.Phil., PGDCA, ADBI is having fourteen years of teaching experience and currently working as Assistant Professor in the Department of Biotechnology and Bioinformatics in Ayya Nadar Janaki Ammal College [Autonomous, CPE, Affiliated to MKU, Re-accredited (3rd Cycle) with A grade with CGPA 3.67 out of 4.0 by NAAC, DBT STAR College] in Sivakasi, Virudhuunagar district. The author received four TNSCST Projects from the Government of Tamil Nadu and organized Workshops and Seminars on Bioinformatics and Proteomics.

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