Evaluation of Larvicidal efficacy of amino acids Schiff base against Aedes aegypti Mosquito Vectors

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ABSTRACT *Derivatives of tyrosine and tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy -2-(4-hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid were prepared and characterized by physical and analytical data, FTIR, ^{1}H NMR, ^{13}C NMR spectra and were screened against larvae of Aedes aegypti.*

Keywords: *Aedes aegypti, larvicidal activity, Aedes albopictus, 5Formylsayicyilic acid -diiodosalicylaldehyde and Tyrosine.*

Introduction

Aedes aegypti is primary vector associated with transmission of globally concerned diseases, Zika, Yellow fever,Dengue and Chikunguniya. The Present study investicats and efficient alternative and comparivive approach for mosquito control which is safe to environment and non target organisams. Mosquitoes are well known for their public health importance as vector for causing dreadful diseases such as malaria, Chikunguniya, Dengue, Zika fever, Yellow Fever .World health organisation received summarised information from 106 malaria endemic countries that about 3.3 Billon people are at risk of malaria causes and 655, 000 malaria deaths were reported [1]. Resently WHO estimated nearly 50-1OO million dengue infections with 20,000 occurring annually world wide, 75 percent of which occurs in Asia pacific region [2].The spreading of these diseases by mosquito is mainly due to ever increasing urbanisation and associated with anthropogenic activities. No effective drug or vaccines is available so far, but control of mosquito population in their breeding sight may directly contributed as an alternative source to prevent these mosquito born diseases .[3] Anti-Dengue Day is observed every year on 15 June to create public awareness about dengue, mobilize resources for its prevention and control and, to demonstrate the Asian region's commitment in tackling dengue fever[4-5]. Dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265–420 AD) Human beings are the primary host of the dengue virus. The dengueviral infection can be acquired via a single bite of mosquitoes. A female *Aedes aegypti* that takes a blood meal from a person infected with dengue fever, during the initial 10-days incubation period, becomes itself infected with the virus in the cells lining its gut. About 10 days later, the virus spreads to other tissues including the *Aedes aegypti* salivary glands and is subsequently released into its saliva. Female *Aedes aegypti* lay eggs on the inner walls of artificial containers. When the small containers fill with water *Aedes aegypti* mosquito larvae hatch from the eggs. After developing four larval stages the larva metamorphoses into pupae. Then pupae is molted into mosquito.The marked spread of dengue virus during and after the Second World War has been attributed to ecologic disruption.. When *Aedes aegypti* carrying dengue virus bites a person, the virus enters the skin together with the *Aedes aegypti* saliva. It binds to and enters white blood cells, and reproduces inside the cells while they move throughout the body of human being. In recent years [6-8], derivatives of amino acid base were found to have potential non-toxic and non-antibiotic resistance of antibacterial, antifungal, mosquito larvicidal[9-20], antiparasitic and anticancer properties. In the present study we have prepared (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoicacid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl)propanoic acid and (IV) 5-(((1-carboxy -2-(4-hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid Sciff bases and have been subjected to in vitro larvicidal activities against larvae of Aedes aegypti.

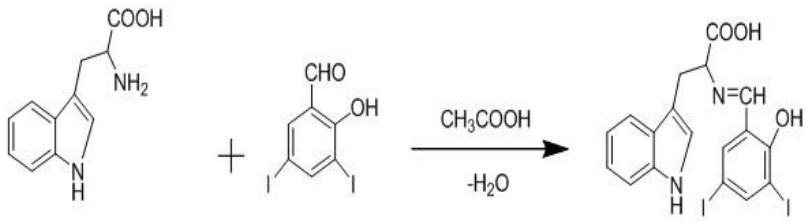
Materials and Methods

Materials

All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrous quicklime for 24 hours, filtered and distilled before use (BP 78°C). Dimethylsulphoxide (sigma) and N, Ndimethylformamide (sigma) were used as such. Thyrosine, tryptophan, 3,5-diiodosalicylaldehyde and 5-formylsalicylic acid were purchased from Alfa Aesar.

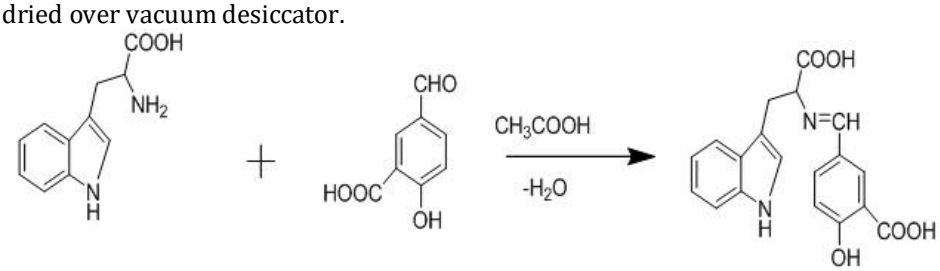
(I) Preparation of 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl) ropanoic acid

2- ((2- hydroxy- 3, 5-diiodobenzylidene)amino)-3-(1Hindol-3-yl)propanoic acid was prepared from equimolar quantity of tryptophan 2.04 g(0.01 mol) and 3,5- diiodosalicylaldehyde 2.73 g(0.01 mol) in 30 ml of methanol were heated at 70°C on refluxed for four hours in oil bath few drops of glacial acetic acid. The crude product were obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol and then dried over vacuum desiccator.



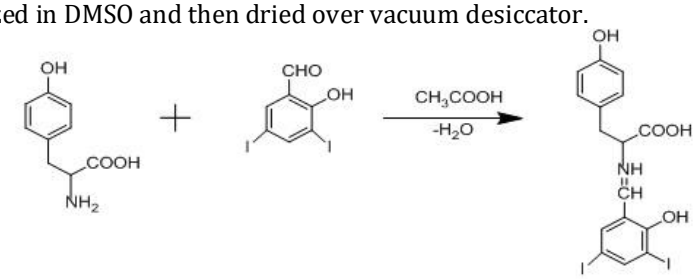
(II) Preparation of 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid

A mixture of 5-formylsalicylic acid 1.66 g (0.01 mol) and tryptophan 2.04 g (0.01 mol) were grained with a pestle in an open mortar at room temperature for 3 minutes. To this reaction mixture sulphuric acid 2 drops and 20 ml DMF were added and refluxed for 4 hours. On completion of reaction as monitored by TLC, the light greenish-colored 5-(((1-carboxy-2-(1Hindol- 3-yl)ethyl)imino)methyl)-2-hydroxybenzoic acid was separated out. The obtained solid was isolated by simple Buchner filtration and was recrystallized from DMF and then dried over vacuum desiccator.



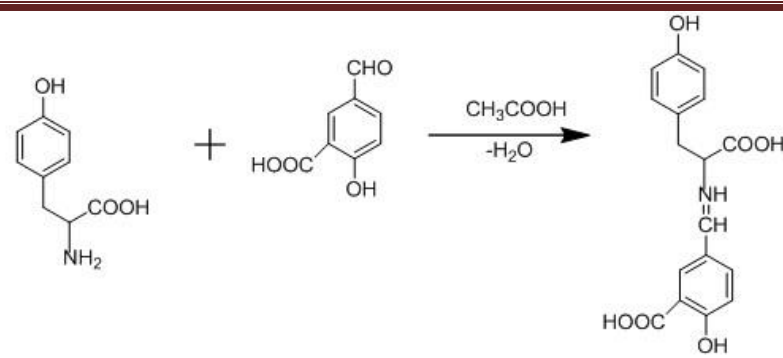
(III)Preparation of 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxyphenyl) propanoic acid

3.6 grams of tyrosine (0.02mol) was mixed with 3.3 g of 3,5-diiodosalicylaldehyde (0.02mol) and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 2-((2-hydroxy -3,5- diiodobenzylidene) amino) -3 - (4-hydroxyphenyl) propanoic acid was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.



(IV) Preparation of 5-(((1-carboxy-2-(4-hydroxy phenyl)ethyl)imino)methyl) -2-hydroxybenzoic acid

A mixture of 5-formylsalicylic acid 1.66g (0.01mol) and tyrosine 1.81 g(0.01mol) were grained in a mortarat room temperature for 10 min.To this reaction mixture sulphuric acid. It two drops and 20ml DMFwere added and refluxed for four hours on compleation pale yellow colour 5- (((1-carboxy-2- (4-hydroxyphenyl) ethyl) imino) methyl)-2-hydroxybenzoic acid was recrystallized using ethanol and then dried over vacuum desiccator.



***Aedes aegypti* rearing**

The *Aedes aegypti* larvae of *Aedes aegypti* and *Aedes albopictus* were collected from National centre for disease control, Government of India ministry of healthand family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The larvae were kept in theplastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Larvicidal bioassay

The larvicidal activity[9-20] of four novel derivatives of tyrosin and tryptophan were assessed by using the standard method as prescribed by WHO. From the stock solution, five different test concentrations (150, 200, 250and 300 ppm was prepared and tested against the freshly moulted (0 – 6 hrs) 4thinstar larvae of *Aedes aegypti* and *Aedes albopictus* DMSO (emulsifier) in water was treated as control. 10 larvae of these *Aedes aegypti* and *Aedes albopictus* species was introduced in 250-ml plastic cups containing 100 ml of aqueous medium (99 ml of dechlorinated water 1ml of emulsifier) and the required amount of four novel derivatives of tyrosin and tryptophan was added. The larval mortality was observed and recorded after 24 hrs of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (Abbott, 1925). The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom was calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 16.0 Version in MS-Excel, 2007.

Results and Discussion

The physical and analytical data of the derivatives tyrosine and tryptophan(I) 2-((2-hydroxy 3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5-(((1-carboxy-2-(1H-indol-3-yl)ethyl) imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2- hydroxy-3,5-diiodobenzylidene)amino)-3-(4-hydroxy phenyl)propanoic acid and (IV) 5-(((1-carboxy-2-(4- hydroxyphenyl)ethyl)imino)methyl)-2-hydroxybenzoic acid are given in Table1.

[I] 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3- (1H-indol-3-yl)propanoic acid

FTIR (cm-1): 3253 & 778 cm-1 (ArO-H), 3082 & 877 cm-1 (-N-H), 1687 cm-1 (R-C=0), 1660 cm-1 (-N==CH), 1381 cm-1 (R-C-OH), 1264 cm-1 (ArO-H) & 607 cm-1 (Ar-I)

1HNMR δ (ppm): 11.0 (s, 1H), 10.1 (s, 1H), 8.65 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.60 (d, 1H), 7.32 (d, 1H), 7.18 (s, 1H), 7.11 (t, 2H), 5.35 (s, 1H), 4.39 (t, 1H) & 3.15; 2.90 (d, 2H)

13CNMR δ (ppm): 177.5 (s), 160.8 (s), 159.1 (s), 147.6 (s), 136.9 (s), 127.7 (d), 123.4 (s), 121.7 (s), 119.8 (s), 118.8 (s), 111.8 (s), 111.1 (s), 86.6 (s), 83.8 (s), 77.9 (s) & 30.8 (s)

[II] 5-(((1-carboxy-2-(1H-indol-3- yl)ethyl)imino)methyl)-2-hydroxybenzoic acid

FTIR (cm-1): 3271 & 841 cm-1 (-N-H), 3181 & 661 cm-1 (ArO-H), 1741 cm-1 (R-C=0), 1705 cm-1 (Ar-C=O), 1660 cm-1 (-N=CH), 1183 cm-1 (R-C-OH) & 1147 cm-1 (Ar-C-OH)

1HNMR δ (ppm): 11.0 (s, 2H), 10.1 (s, 1H), 8.65 (s, 1H), 8.32 (s, 1H), 8.12 (d, 1H), 7.60 (d, 1H), 7.32 (d, 1H), 7.23 (d, 1H), 7.18 (s, 1H), 7.11 (t, 2H), 5.35 (s, 1H), 4.39 (t, 1H) & 3.15; 2.90 (d, 2H)

13CNMR δ (ppm): 177.5 (s), 171.8 (s), 164.5 (s), 160.8 (s), 136.5 (s), 135.8 (s), 132.3 (s), 130.9 (s), 127.7 (s), 123.4 (s), 121.7 (s), 119.8 (s), 118.8 (s), 112.1 (s), 111.8 (s), 77.9 (s) & 30.8 (s)

[III] 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3- (4-hydroxyphenyl)propanoic acid
FTIR (cm-1): 3451 & 607 cm-1 (ArO-H), 1696 cm-1 (R-C=0), 1660 cm-1 (-N=CH), 1480 cm-1 (R-C-OH) & 517 cm-1 (Ar-I)

1HNMR δ (ppm): 11.0 (s, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.12 (d, 2H), 6.70 (d, 2H), 5.35 (s, 1H), 4.39 (t 1H) & 3.27; 3.02 (d, 2H)
13CNMR δ (ppm): 177.5 (s), 160.8 (s), 159.1 (s), 155.7 (s), 147.6 (s), 136.9 (s), 130.5 (s), 130.2 (s), 127.8 (s) 115.8 (s), 88.6 (s), 83.8 (s), 70.8 (s) & 38.1 (s)

[IV] 5-(((1-carboxy-2-(4-hydroxyphenyl) ethyl)imino)methyl)-2-hydroxybenzoic acid
FTIR (cm-1): 3208 & 661 cm-1 (ArO-H), 1705 cm-1 (R-C=0), 1696 cm-1 (Ar-C=O), 1660 cm-1 (-N=CH), 1345 cm-1 (-N=CH), 1246 cm-1 (R-C-OH) & 1183 cm-1 (Ar-OH)
1HNMR δ (ppm): 11.0 (s, 2H), 8.32 (s, 1H), 8.12 (d, 1H), 811 (s, 1H), 7.23 (d, 1H), 7.12 (d, 2H), 6.70 (d, 2H), 5.35 (s, 2H), 4.39 (t, 1H) & 3.27; 3.02 (d, 2H)
13CNMR δ (ppm): 177.5 (s), 171.8 (s), 164.5 (s), 160.8 (s), 155.7 (s), 135.8 (s), 132.3 (s), 130.5 (s), 130.2 (s), 118.0 (s), 115.8 (s), 112.1 (s), 70.8 (s) & 38.1 (s)

Table. 1 -The physical and analytical data of the derivatives of tyrosine and tryptophan

Derivatives of Amino acids	Molecular Weight	Nature of Appearance	% of yield	Elemental Analysis (in %)				
				C	H	O	N	I
[I] C18H14I2N2O3	560.12	Yellow Crystalline Solid	88	38.60	2.52	8.57	5.00	45.31
[II] C19H16N2O5	352.34	Yellow Crystalline Solid	81	64.77	4.58	22.70	7.95	-
[III] C16H13I2NO4	537.08	Yellow Crystalline Solid	76	35.78	2.44	11.92	2.61	47.26
[IV] C17H15NO6	329.30	Pale Crystalline Solid	69	62.00	4.59	29.15	4.25	-

Table 2. Larvicidalactivity of derivatives of Tyrosin and Tryptophan are determined as recommended by WHO in 50,200, 250 and 300 ppm concentration in dimethyl sulfoxide(DMSO) solvent. The results of larvicidal activity of (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5- (((1-carboxy-2-(1H-indol-3-yl) ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)-3-(4-hydroxyphenyl) propanoic acid and (IV) 5-(((1-carboxy -2-(4-hydroxyphenyl)ethyl)imino) methyl)-2-hydroxybenzoic acid.The larvicidal activity of I-IV (Table2) clearly indicate that all the compounds control the growth of larvae. The nature of bonding and structure of azomethine organic compounds are elucidated as reported in the literature by the elemental analysis, melting point, FTIR, 1HNMR, 13CNMR, spectral analysis, chromatography and molar ratio methods. In accordance with the data obtained in the present investigation, it is found that the larvicidal activity of (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5- (((1-carboxy-2-(1H-indol-3-yl) ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)-3-(4-hydroxyphenyl) propanoic acid and (IV) 5-(((1-carboxy -2-(4-hydroxyphenyl)ethyl)imino) methyl)-2-hydroxybenzoic acid.Increases depent upon the functional groups present in the derivatives of amino acides Schiff bases (III < I< II< IV).

Table.. Larvicidal activity of derivatives of tyrosin and typtophan

Compounds	Con. (ppm)	Larval mortality	95% Confidence Limits (ppm)		
			LC50 (LCL-UCL)	LC90 (LCL-UCL)	χ2
I	150	27.23±3.50	177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539
	200	41.21±3.24			
	250	67.30±3.30			
	300	88.20±2.20			
II	150	21.20±4.50	209.58 (192.10-227.86)	379.01 (342.85-434.11)	2.509
	200	33.40±3.50			
	250	56.30±4.30			

	300	79.40±1.20			
III	150	23.20±1.30			
	200	32.10±2.20	220.45 (200.61-242.69)	417.14 (370.09-494.14)	2.654
	250	51.50±3.40			
	300	74.30±2.44			
IV	150	21.20±3.20			
	200	37.27±4.30	177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539
	250	57.60±2.30			
	300	79.50±2.30			

Values are mean ± S.D of five replication; Number of larvae =10; LC50=Lethal concentration 50 and LC90=Lethal concentration 95; Values with different alphabet in column are statistically significant ($p<0.05$ level; DMRT).

The role of juvenile hormone in mosquito development and reproduction

Derivatives of Tyrosin and Tryptophan (I-IV) act as a Juvenile Hormone, growth regulator like methoprene analog against larvae of a Aedes aegypti when used as larvicides[9-12].Juvenile Hormone plays an antimetamorphic role during development, maintaining the status quo in preimaginal stages and preventing immature *Aedes aegypti* from precociously turning into adults. Juvenile Hormone are high while the larva is feeding and growing, but drop to permit metamorphosis. This hypothesis has not been tested in *Aedes aegypti*. The functions of Juvenile Hormone during preimaginal stages in *Aedes aegypti* have been mostly extrapolated from studies on other mosquitos or deduced from the phenotypic changes induced by topical application of Juvenile Hormone or its analogues.

Derivatives of Tyrosin and Tryptophan schiff's bases blocks the embryonic development of *Aedes aegypti* and inhibit egg hatching. Juvenile Hormone A also interfere with metamorphosis and prevent the emergence of adults. They are particularly effective against 4th instar mosquito larvae. A majority of the Derivatives of Tyrosin and Tryptophan -treated larvae died during the pupal stage. Larval exposure to Juvenile Hormone A affects metamorphic midgutre modelling. Themetamorphic midgutre modelling includes two processes, formation of pupal/adult midguts through the division and differentiation of imaginal diploid cells, and programmed death of larval polytene midgut cells treating 4th instar *Aedes aegypti* larvae with derivatives of Tyrosin and Trptophan blocks degeneration of larval midgut epithelium. Derivatives of Tyrosin and Tryptophan -treated larvae pupae, but the pupae developed from treated larvae contain two midgut epithelial layers, larval midgut and the pupal/adult midgut. This Derivatives of Tyrosin and Tryptophan effect is presumably achieved through modulating the action of ecdysterone (20E), as the derivatives of Tyrosin and Tryptophan treatment causes dysregulation of many genes involved in the stage-specific response to ecdysterone (20E), including ecdysone receptor. Organisms are subject to "trade-offs" between the energetic demands of reproduction and the energy required to survive. JH is the central hormonal regulator of life-history trade-offs in many mosquitos, including *Aedes aegypti. Aedes aegypti* must not only allocate nutrients properly within each developmental stage but must also consider the effects of immediate resource allocations on future reproduction and overall fitness. Three major periods can be defined in the development of the ovaries during a gonotrophic cycle in *Aedes aegypti* mosquitoes: by apoptosis. By resorbing excess reproductive tissues, mosquitoes can alter previous reproductive decisions by redirectingresources away from reproduction in favour of competing physiological activities. Under this model, excessive allocations toward somatic physiology or reductions in incoming nutrition often result in reductions to reproductive output. The developmental fate of the remaining follicle is also sensitive to nutrition and Juvenile Hormone. Nutrients and transcripts related to VG accumulated in *Aedes aegypti* treated with the Juvenile Hormone analogue derivatives of Tyrosin and Tryptophan or fed 20% sucrose. These mosquitoes have increased fecundity and decreased follicle resorption after a blood meal. In addition to nutrition, ating triggers many changes in female mosquitoes that enhance reproductive success. Many of the phenotypic, molecular and biochemical changes observed when manipulating nutrition and hormonal status Juvenile Hormone A- sterilizing effects have been described as mediated by suppressing the expression of ecdysone-regulated genes in female *Aedes aegypti* stressing the importance of the endocrine balance between Juvenile Hormone and ecdysterone (20E), during the gonotrophic cycle. It is concluded that the increase in the larval mortality of *Aedes aegypti* and *Aedes albopictus* depend upon the functional groups present in the Schiff bases
(III< II< IV< VI< I< V). Table 2.

Conclusion

The derivatives of Tyrosin and Tryptophan (I) 2-((2-hydroxy-3,5-diiodobenzylidene)amino)-3-(1H-indol-3-yl)propanoic acid, (II) 5- (((1-carboxy-2-(1H-indol-3-yl) ethyl)imino)methyl)-2-hydroxybenzoic acid, (III) 2-((2-hydroxy-3,5-diiodobenzylidene) amino)-3-(4-hydroxyphenyl) propanoic acid and (IV) 5-(((1-carboxy -2-(4-hydroxyphenyl)ethyl)imino) methyl)-2-hydroxybenzoic acid were prepared and were screened against larvae of *Aedes aegypti*. It was concluded that the increase in the larval mortality of *Aedes aegypti* depend upon the functional groups present in the Schiff bases.

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