LARVICIDAL STUDY OF PURINE DERIVATIVES

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Abstract: ***Purine derivative Schiff bases 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol and N-(furan-2-ylmethylene)-9Hpurin-6-amine obtained by the condensation of adenine with 3,5-diiodosalycylaldehyde and furfural have been synthesized and characterized by spectroscopic studies viz., Fourier-transform infrared, proton Nuclear Magnetic Resonance. The synthesized compounds have been tested against the larvae of Aedes aegypti mosquitos and have significant larvicidal activity.***

Keywords: Larvicidal activity, Aedes aegypti, dengue fever and adenine

I. INTRODUCTION

Numerous diseases are transmitted by mosquitoes to human than any other group of arthropods. Therefore, Mosquitoes as "public enemy number one"[1] affirms by World Health Organisation. They act as a flight path for most of the life aggressive diseases like West Nile virus infection, encephalitis, malaria, chikungunya fever, yellow fever, dengue fever, filariasis *etc.*, in almost all tropical and subtropical countries and many other parts of the world. The Zika virus disease is also transmitted by *Aedes. Aegypti* and it has spread rapidly within the Americans after an occurrence in Brazil in 2014. In 2015, an increasing number of infants with small head circumference, "microcephaly", was observed in Brazil's Northeast region[2]. In the year 2016, the estimated cases of Zika virus ranged between 4,40,000 and 13,00,000 in the country. Brazil accounts for 94% of confirmed cases of Zika virus, then Then Americans, according to the Pan American Health Organization (2016)[3]. In January 2017, the Brazil Ministry of Health reported 12 suspected cases of yellow fever, another viral disease transmitted by *Aedes. aegypti*, from six municipalities in rural areas in the State of Minas Gerais. Later, after an update, a total of 110 suspected cases, including 30 deaths, had been reported from 15 municipalities of Minas Gerais. The serological tests for 19 suspected cases were positive for yellow fever which included 10 deaths[4].

Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[5]. However, indiscriminate use of these larvicidal has disrupted natural biological control systems and led to resurgence of mosquitoes [5] and has often resulted in widespread development of resistance [7]. Juvenile Hormones are secreted by a pair of endocrine glands behind the brain called the corpora allata. Which are important for the production of eggs in female *Aedes aegypti* mosquitoes. They were discovered in 1965 by Williams and Slama and the first molecular structure was solved in 1967. Juvenile hormones (JH) are a group of acyclic sesquiterpenoids that regulate many aspects of mosquitoes physiology[8-10].

II. EXPERIMENTAL PROCEDURE

2.1 Materials

Solvents used in this study were purified and dried according to standard procedures. Adenine, 3,5-diiodosalicylaldehyde and furfural were obtained from Merck specialities Ltd., Mumbai, India and all chemical pure and AR grade.

2.2 Preparation of 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol

2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol was prepared by condensation of adenine with 3,5-diiodosalicylaldehyde. Equimolar (0.5 m mol) quantity of 3,5-diiodosalicylaldehyde and adenine was dissolved in 20 mL of ethanol and the solution was refluxed for 4 h under constant stirring. This condensation reaction was carried out by using acid catalyst (few drops of glacial acetic acid). The formed water was removed from the reaction mixture using sodium sulfate (dehydrating agent). After completion of the reaction, the mixture was reduced to half of its original volume using a water bath and kept aside at room temperature. Yellow crystals of ligand were obtained from slow evaporation (Yield: 83%). The Schiff base is characterized by FTIR and Proton NMR spectroscopy.

2.3 Preparation of N-(furan-2-ylmethylene)-9Hpurin-6-amine

N-(furan-2-ylmethylene)-9Hpurin-6-amine was prepared by condensation of adenine with furfural. Equimolar (0.5 m mol) quantity of furfural and adenine was dissolved in 20 mL of ethanol and the solution was refluxed for 4 h under constant stirring. This condensation reaction was carried out by using acid catalyst (few drops of glacial acetic acid). The formed water was removed from the reaction mixture using sodium sulfate (dehydrating agent). After completion of the reaction, the mixture was

reduced to half of its original volume using a water bath and kept aside at room temperature. Yellow crystals of ligand were obtained from slow evaporation (Yield: 79%). The Schiff base is characterized by FTIR and Proton NMR spectroscopy.

2.4 *Aedes aegypti* rearing

The larvae of *Aedes aegypti* were collected from National Centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The larvae were kept in the plastic 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 formation on the water surface.

2.5 Larvicidal bioassay

The larvicidal activities of two novel derivatives of adenine were assessed by using the standard method as prescribed by World Health Organization. From the stock solution, five different test concentrations (150, 200, 250 and 300 ppm was prepared and tested against the freshly molted (0 – 6 hrs) 4th instar larvae of *Aedes aegypti* DMSO (emulsifier) in water was treated as control. Ten larvae of these *Aedes aegypti* 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 six novel derivatives of adenine 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[12]. The LC50, LC90, 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.

III. RESULTS AND DISCUSSION

3.1 FTIR Spectra

The vibrational spectra provides valuable information regarding the nature of functional group of the 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol and N-(furan-2-ylmethylene)-9Hpurin-6-amine. The IR spectra of the Schiff bases derived from purine showed in **Fig 1 & 2.** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol having a broad band at 3568 cm^{-1} & a sharp peak at 690 cm^{-1} indicates hydroxyl group in benzylidine ring, a broad band at 3235 cm^{-1} & a sharp peak at 877 cm^{-1} shows amide group present in purine ring, a sharp peak at 1645 cm^{-1} confirms the presence of azomethine, a sharp peak at 1615 cm^{-1} refers to the presence of secondary ketimine in purine ring, a medium peak at 1147 cm^{-1} indicates aromatic carbon with hydroxyl oxygen in benzylidine ring and a medium to sharp peak obtained at 643 cm^{-1} shows aromatic carbon with iodine in benzylidine ring. N-(furan-2-ylmethylene)-9Hpurin-6-amine having a broad band at 3270 cm-1 & a sharp peak at 740 cm^{-1} refers to amide group in purine ring, a sharp peak at 1650 cm^{-1} confirms the presence of azomethine, a sharp peak at 1570 cm^{-1} shows secondary ketimine group in purine ring and a sharp peak obtained at 1230 cm^{-1} indicates aromatic carbon with aromatic oxygen in furan ring.

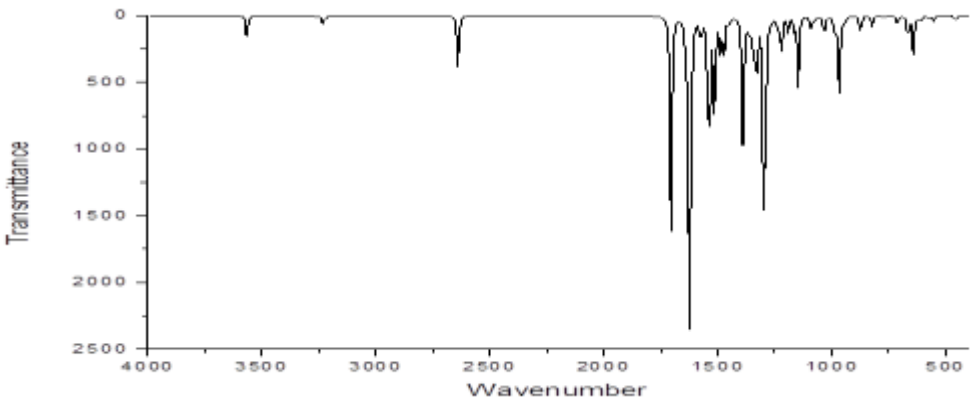

Fig 1. FTIR Spectral Evidence of 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol

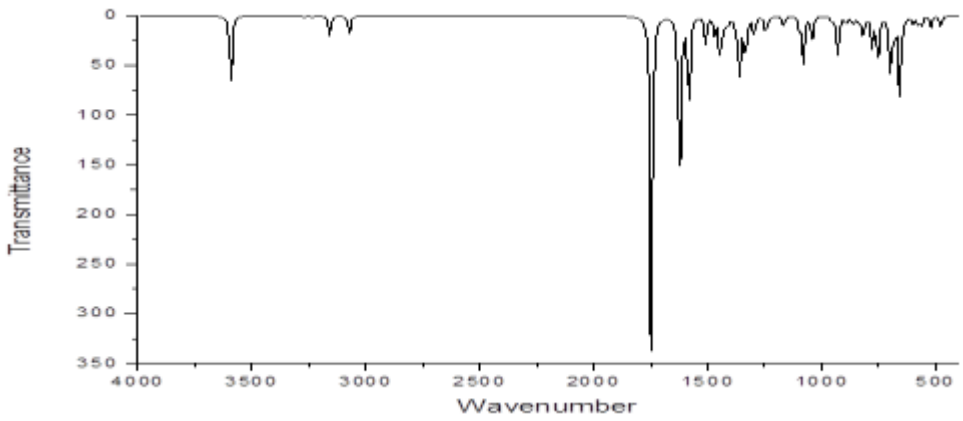

Fig 2. FTIR Spectral Evidence of N-(furan-2-ylmethylene)-9Hpurin-6-amine

3.2 ^{1}HNMR

The [1]H NMR spectra results given in the **Fig 3 & 4** confirm the presence of compounds show 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol and N-(furan-2-ylmethylene)-9Hpurin-6-amine. 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol having a broad band at 15.18 ppm indicates hydroxyl proton in benzylidine ring, a sharp singlet peak appears at 13.65 ppm shows amide proton in purine ring, a sharp singlet peak at 8.88 ppm designates a single proton in purine ring, a sharp peak at 8.71 ppm confirms azomethine proton, a medium singlet peak at 8.57 ppm shows a proton in purine ring, a sharp singlet peak at 8.07 ppm indicates a proton in benzylidine ring and a sharp singlet peal at 7.92 ppm refers a proton in benzylidine ring. N-(furan-2-ylmethylene)-9Hpurin-6-amine having a sharp singlet peak at 13.65 ppm shows amide proton in purine ring, a sharp singlet peak at 8.88 ppm indicates presence of a single proton in purine ring, a medium singlet peak at 8.57 ppm refers a single proton in purine ring, a sharp singlet peak at 8.10 ppm confirms the presence of azomethine proton, a doublet peak at 7.84 ppm (J=8.4) indicates a single proton in furan ring, another doublet peak at 6.93 ppm (J=13.8) shows a proton in furan ring and a triplet peak at 6.63 ppm (J=16.8) refers a single proton in furan ring.

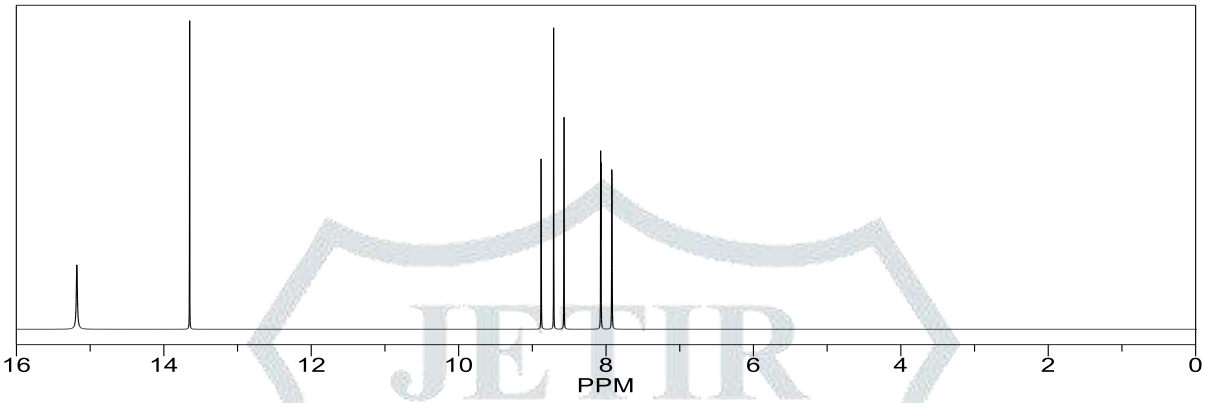

Fig 3. [1]NMR Spectral Evidence of 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol

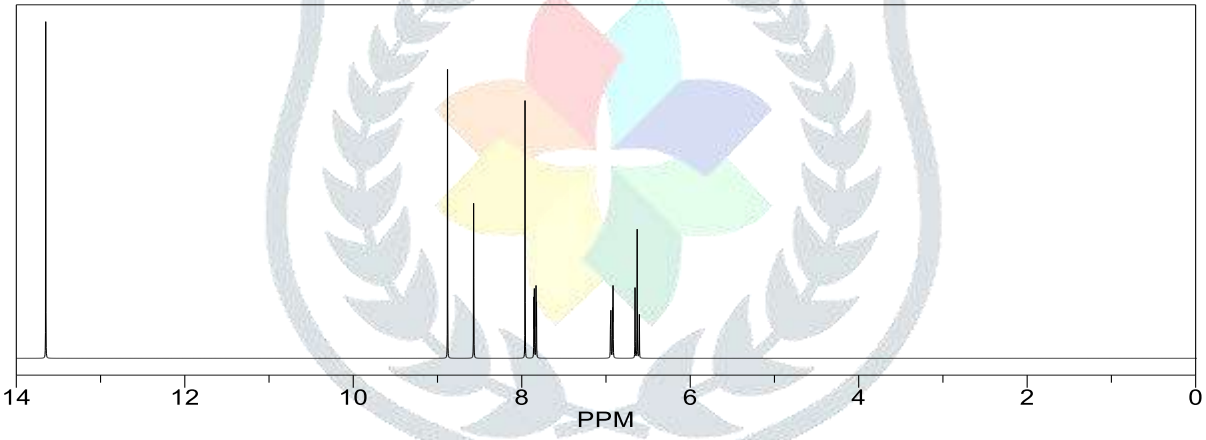

Fig 4. 1NMR Spectral Evidence of N-(furan-2-ylmethylene)-9Hpurin-6-amine

3.3 Larvicidal Study

Larvicidal activity[11-21] of all azomethine compounds are determined as recommended by World Health Organization in 150, 200, 250 and 300 ppm concentration in dimethyl sulfoxide(DMSO) solvent. Juvenile hormone is secreted by a pair of endocrine glands behind the brain called the corpora allata. They are also important for the production of eggs in female *Aedes aegypti* mosquitoes. They regulate development, reproduction, diapause, and polyphenisms. In mosquitoes, Juvenile hormones which ensure growth of the larvae, while preventing metamorphosis. Because of their rigid exoskeleton, mosquitoes grow by successively shedding successfully. It is a process known as molting. Derivatives of adenine act as a Juvenile Hormone, like methoprene analog against larvae the growth regulator when used as an larvicides. Most insect species contain only juvenile growth hormone III. To date Juvenile hormones, Juvenile hormones I, and Juvenile hormones II have been identified only in butterflies and moths Lepidoptera.

The form Juvenile hormones B3 appears to be the most important Juvenile hormones in the diptera or flies. Certain species of crustaceans have been shown to produce and secrete methyl farnesoate, which is juvenile hormone III lacking the epoxide group. Methyl farnesoate is believed to play a role similar to that of Juvenile hormones in crustaceans. Being a sesquiterpenoids, Juvenile hormones chemical structure differs significantly from the structure of other animal hormones. Some Juvenile hormones analogues have been found in conifers.

Table 1. Larvicidal activity of derivatives of adenine against larvae of *Aedes aegypti*

Compounds	Con. (ppm)	Larval mortality	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
	150	21.30±1.23			
I	200	34.20±2.33	210.48 (101.29-370.07)	381.19 (284.07-1523.71)	5.997
I	250	52.30±2.32	210.48 (101.29-370.07)	381.19 (284.07-1523.71)	5.997
I	300	81.20±2.20	210.48 (101.29-370.07)	381.19 (284.07-1523.71)	5.997
I	150	27.23±3.50	210.48 (101.29-370.07)	381.19 (284.07-1523.71)	5.997
II	200	41.21±3.24	177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539
II	250	67.30±3.30	177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539
II	300	88.20±2.20	177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539
II			177.39 (159.91-193.45)	331.85 (303.48-373.20)	2.539

Values are mean ± S.D of five replication; Number of larvae =10; LC_{50}=Lethal concentration 50 and LC_{90}=Lethal concentration 95; Values with different alphabet in column are statistically significant (p<0.05 level; DMRT

Derivative of purine 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol and N-(furan-2-ylmethylene)-9Hpurin-6-amine were against larvae of female *Aedes aegypti* mosquitoes results shown in **Table 1**. Both compounds have very good Larvicidal activity gradually increasing based on their different concentrations i.e., 1.5 times increase in addition of 50 ppm concentrated solution.

IV. CONCLUSIONS

In the present research studies, our efforts were to synthesis and characterize purine derivative compounds by condensation method. These synthesized compounds were characterized by various physicochemical and spectral analysis. The compounds were tested against larva of female *Aedes aegypti* mosquitoes. Both purine derived compounds were 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol and N-(furan-2-ylmethylene)-9Hpurin-6-amine having good Larvicidal activity against tested organism. Compound N-(furan-2-ylmethylene)-9Hpurin-6-amine showed slight higher activity against larvae of female *Aedes aegypti* mosquitoes than 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol.

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REFERENCES

[1] World Health Organization. *Report of the WHO informal consultation on the evaluation on the testing of insecticides*, CTD/WHO PES/IC/96.1. Geneva: WHO; 1996. p. 69.

[2] Marinho F, Araújo VEM, Porto DL, Ferreira HL, Coelho MRS *et al.* Microcephaly in Brazil: Prevalence and characterization of cases from the Information System on Live Births (Sinasc), 2000–2015. *Epidemiol Serv Saúde* 2016; *25*(4): 1–11.

[3] *Pan American Health Organization (PAHO), Zika–Epidemiological Report Brazil (2016).* Available from: *http://www.paho.org/hq/index.php?option=com_docman&task=doc_view& gid=35221 &&Itemid=270* (Accessed on January 17, 2017).

[4] *Emergencies preparedness, response. Yellow fever–Brazil: Disease outbreak news.* Geneva: World Health Organization 2017. Available from: *http://www.who.int/csr/don/13-january-2017-yellow-fever-brazil/en/* (Accessed on January 17, 2017).

[5] *PENG Z, YANG J, WANG H*. Simons FER Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. Insect Biochem Mol Biol 1999; 29: 909-914.

[6] Croft, B.A.; Brown, A.W.A. Responses of arthropod natural enemies to insecticides. *Ann. Rev. Ent.*1975, *20*, 285-335.

[7] World Health Organization (WHO). *Vector Resistance to Pesticides, Technical Report Series 818*;WHO: Geneva, Switzerland, 1992.

[8] Abhilash PC, Singh N. An Indian scenario Journal of Hazardous Materials.; 165(1):1. (2009)

[9] Ahmad M. Pakistan Journal of Crop Protection.; 27:1367.(2008)

[10] Ahmad M. Journal of Agricultural Research.; 45(4):319.(2007)

[11] Elumalai, D., Hemalatha, P., Kaleena, P.K, ., *Journal of the Saudi Society of Agricultural Sciences.4:7(2015).*

[12] Abhilash PC, Singh N. An Indian scenario Journal of Hazardous Materials.; 165(1):1. (2009)

[13] Ahmad M. Pakistan Journal of Crop Protection.; 27:1367.(2008)

[14] Ahmad M. Journal of Agricultural Research.; 45(4):319.(2007)

[15] Anshul N, Bhakuni RS, Gaur R, Singh D. Florida Entomology.; 96:897.(2013).

[16] Bami HL. Chemical Weekly.; 4:7.(1997).

[17] Baskar K, Kingsley S, EzhilVendan S, Paulraj MG, Duraipandiyan V, Ignacimuthu S. Chemosphere.; 75:355.(2009).

[18] Walker, A. N.; Bush, P.; Puritz, J.; Wilson, T.; Chang, E. S.; Miller, T.; Holloway, K.; Horst, M. N. *Integrative and Comparative Biology*. **45** (1): 118.(2005).

[19] Yang Y, Li Y, Wu Y. Journal of Economic Entomology.; 106(1):375.(2013).

[20] Subramonithangam T, Kathiresan K. Journal of Current Science.; 57:914.(1988).

[21] Saleem MA, Ahmad M, Aslam M, Sayyed AH. Pakistan Journal of Ecological Entomology.; 101(5):1667.(2008).