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Larvicidal Activity of Celosin A, A Novel Compound Isolated from Celosia Argentea Linn

B. Ramesh[1], V. Vanita Das[2]*
Department of Zoology,University College of Science,
Osmania University,Tarnaka, Hyderabad,
Telangana-500007, India.

Abstract:-Mosquitoes are the most dangerous diseases spreading vector and one of the important vector is *Aedesaegypti*. Natural products act as toxic agents and its active principles as an alternative mosquito control strategy was available from ancient times. These are nontoxic, easily available at affordable prices, biodegradable and show broad-spectrum target-specific activities against different species of vector mosquitoes. Pharmacological and Phytochemistry uses of *Celosia argentea Linn* shown health benefits of including anti-oxidant, anti-diarrhoeal, Anti-diabetic, nutritive, bile juice increase and use as blood Tonic. The phytochemical evaluation of whole plant of *Celosia argentea* was examined but larvicidal (*Aedesaegypti*) activity remains unknown. In this article, we explored the larvicidal activity of *Celosia argentea*(leaf and root extracts), its active principle Celosin A (isolated from leaf and root). Lethal concentration (LC50) was against fourth instar larvae of Aedesaegypti by leaf (LC 10.66ppm) and root (LC50 1.8ppm). Interestingly, Celosin A is the major (45%) compound isolated from leaf and root extracts, which shown the LC50 1.488ppm. For the first time, we studied the Celosin A chemical nature against mosquito and it can be a future drug or toxicant against *Aedesaegypti*larva.

I. INTRODUCTION

Mosquitoes, which are a nuisance for millions of people globally, can carry different infections than any other group of arthropods. WHO has labelled mosquitoes "public enemy number one." More than 700,000,000 people globally, including 40,000,000 Indians, are affected by mosquito based infections each year, which are very prevalent in more than 100 countries. They act as a vector for various severe diseases as malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, etc. in virtually all tropical and subtropical countries as well as many other regions of the world. Raising environmental standards and fostering high-genic conditions can minimise mosquito reproduction in developing countries, ultimately halting the development of vector-borne diseases. The only alternatives for managing infections spread by mosquitoes are substances containing organochlorine and organophosphate. 2001; J.M. Kabaru and L. Gichia. This hasn't been particularly successful, though, because of issues with the economy, the environment, technology, operations, and people (Elumalai et al., 2013). In recent years, operations to control mosquitoes have used fewer of the previous synthetic insecticides. This is because there are not many new pesticides, synthetic pesticides are expensive, environmental safety is an issue, they are not good for human health and other humans, they are not biodegradable and biodegradable from higher ecosystems, and pesticides are also dispersed around the world. The Environmental Protection Act of 1969, established laws and regulations to limit the use of controlled substances in nature (Gbolade, A., 2000). Due to the need for effective and transparent mosquito control, researchers are seeking alternative methods that prioritize public awareness, monitoring and evaluation, reduction and environmentally friendly minimally toxic larva control (Hahn, C.S. et al., 2001). has led to the need to find pesticides that are environmentally friendly, economically viable, biodegradable and mosquitoes (Kamaraj et al., 2008). Accordingly, the application of environmental benefits, such as control of the health of vectors, has replaced the use of pesticides as the mainstay of management (Abbott, W.S., 1925).

Two different methods have been developed to study biodiversity and effectively manage many mosquito species in the field of insecticide from plants (Arivoli, S. and Samuel, T., 2011). The individual isolates contain a combination of plant-organic compounds that work synergistically in behavioural and physiological functions. Therefore, pests are unlikely to develop resistance to these chemicals. The availability of highly effective, suitable and ecologically appropriate biopesticides is essential for effective vector control. Botanicals are used as pesticides due to its excellent antibacterial properties. Finally, they can be used to prevent mosquitoes. And Abbott, W.S., 1925, listed more than 3000 plant species with high antibacterial properties. Cantrell et al. (2014) investigated different types of larvicidal plants, including isolation, growth and reproduction inhibitory phytochemicals, botanical ovicides, synergistic with admixture, additive and antagonistic combinations, residual capacity, Effects, prevention and analysis of non-targets. illness.

II. EXPERIMENT

A. Preparation of Cassia auriculata, Celosia argentea extracts.

The *Celosia argentea* plant was identified and validated by taxonomist Prof.Ramchandra Reddy at the Department of Botany at Osmania University Hyderabad (Deposited Number: Bot/143/OU/A.0693/HYD). The leaves and roots of the Celosia argentea plant were collected from the Kurnool district of Andhra Pradesh, Nallamalla forest. The fresh leaves were mechanically milled into coarse powder at room temperature after being shade dried for 6 to 8 days. The dry powder leaf extract prepared in high polarity organic solvent methanol (CH3OH), as described by

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Anandharajan et al. in 2006, Dheeba et al. in 2010, Amit in 2011, and Kothari et al. in 2011 using dried powder (500 g) of leaves and roots. In order to prevent evaporation, solvent extracted solutions were made in clean bottles and left at room temperature for 72 hours. Total reaction mixture was filtered via 0.45µm filter paper after 72 hours. To get rid of any remaining solvent, these (root and leaf) filtered plant extracts were extracted and concentrated using a rotary evaporator (Superfit TM). *Celosia argentea* concentrated preparation was kept at -20˚C until usage. The yield for 500g of dry powder was 21g (leaf) and 9g (root) of methanol extracts from *Celosia argentea*, respectively.

B. Larval susceptibility tests

Five replicates were used in each of the three trials that made up the entire set of assays against vector mosquitoes. By combining 100 mg of crude extract with 1 ml of deionized water, four distinct extract stock solutions (1000 ppm) were created. The volume was then increased to 100 ml with distilled water. Following the release of 25 fourth instar larvae at various dilutions of 0.01 ppm, 15 ppm, 25 ppm, 50 ppm, and 100 ppm from the stock solution, mortality was measured after 24 and 48 hours. The beakers were kept in a room with a constant temperature of 28°C and 2°C, with the larvae acting as a control. Five replications of each treatment were carried out (Tonk et al., 2006). The testing for larval susceptibility were conducted in accordance with the established WHO protocol (WHO, 2005). The following approach was used to test each test solution's larvicidal ability on an Ae. aegypti larva in its fourth instar. Larvae of the fourth instar were released in 200ml of plant extract mixture, and parallel control trials without extract were conducted. After being exposed to each solution for 24 and 48 hours, the number of departed larvae was counted, and the % mortality was calculated using the average of five repetitions. When control mortality varied between 5% and 20%, mortality was noted and corrected using Abbott's (1925) formula. According to Busvin (1971), LC50 values of plant extract from Ae. aegypti were determined based on % mortality values by computing the regression line using Finney's (1971) probit analysis.

C. Analysis of plants extract by TLC

Thin Layer Chromatography silica coated on aluminium sheets were prepared as per the literature and pinch of ethanol extract was placed on tightly packed plate. The extracts were ran using chloroform and Methanol (9:1) combination, and separated were analysed based on its *rf* value.

$$Rf = \frac{\text{Distance moved by analytes from origin}}{\text{Distance moved by solvent front from origin}}$$

D. Statistical analysis

The LC90, LC50 calculation and larval mortality data were analyzed using probit tool and data stats at 95% fiducial with upper confidence and lower confidence level were calculated with SPSS11 software tool.

III. RESULTS AND DISCUSSION

A. Phytochemical analysis of leaf and root extract by TLC

Phyto-chemical analysis of the whole plant extract revealed that the plant extract contains phenols, terpenes, triterpenes, coumarins etc. Methanol extracts were found to be active on several plants and TLC was performed. TLC of the methanol extract shown 8 bands with Rf values of 0.95(triterpenoids), 0.81 (steroids), 0.69 (catechincosic), 0.58 (phenols), 0.46 (glycosides), 0.38 (saponins), 0.06 (terpene) and 0.04 (quercetins).

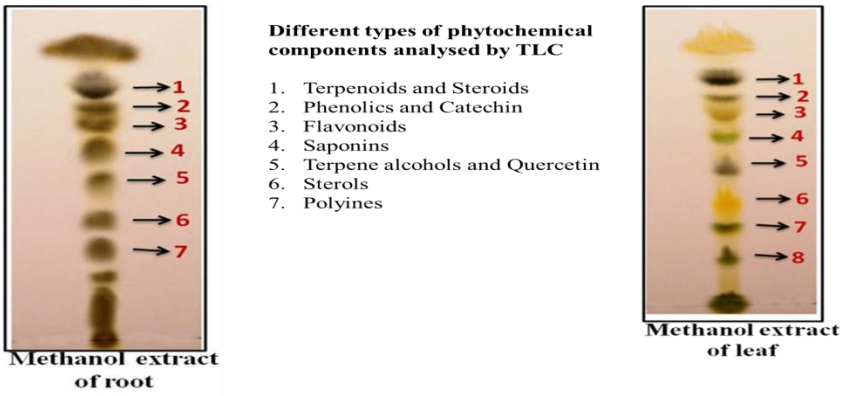

Fig. 1: Phytochemical analysis of leaf and root by TLC. Isolation of bioactive compounds in methanolic extract of Cockscomb whole plant using thin layer chromatography.

B. Larvicidal Bioassay of leaf and root extracts.

The bioassay evaluation of the methanolic extract of *Celosia argentea* (leaf and root) ranged from 2.5 ppm/L to 40ppm/L. All larval bioassay tests with two different (leaf and root) extracts showed that the mortality rate increased with increasing. When the tested extracts were compared, the greatest activity was found against 4th instar larvae of *Aedesaegypti*, LC50 is shown in the table. 1 and Table 2. Simultaneously, LC50 values were recorded in larval bioassays of *Aedesaegypti*. Figures 2 and 3 show graphical representations of *Aedesaegypti* larval mortality. Probit kill percentage is evaluated according to the importance of the probit kill Finney table.

Secondary metabolites have evolved to protect plants from herbivores and are often toxic to species. These secondary metabolites expose the insect to toxins that affect multiple molecular targets when ingested by the insect. Proteins (such as enzymes, receptors, signalling molecules,

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ion channels, and structural proteins), nucleic acids and biomembranes, and other cellular components are some of the targets. In turn, this has many effects on the physiology of different receptor sites, the most important of which are abnormal in the brain (for example, participation in the signal transduction layer standard, receptor activation and function, neurotransmitter production, storage, release, binding, and enzymes obtained from Rattan, plant secondary metabolites of bacteria. studied the action process and noted that it affects the body of many, including inhibition of acetylcholinesterase (essential oil), GABA-gated chloride channels (Lifenol), sodium and potassium ion exchange damage (pyrethrins). and inhibition of cellular respiration (via rotenone). Its action of blocking calcium channels (ryanodine), meningeal function (sabatia) and octopamine receptors (thymol) has a significant effect on hormonal balance, mitotic poisoning (azadirachtin), morphogenesis and other molecular events. Acetylcholinesterase (AChE), an important enzyme that inhibits the transmission of nerve impulses through synaptic pathways, has been shown to be resistant to organophosphates and carbamates. Regulation of AChE is known as one of the main defense mechanisms of pests.

Table 1: Determination of LC 50 *Celosia argentea*leaf extract

S. No	Conc. of *Celosia argentea* Leaf extract In ppm	Log Concentration	Percent of Kill (LC50)	Probit Kill Finney Table
1	**0**	**0**	**0**	**0**
2	**2**	**0.3010**	**2.5**	**3.87**
3	**4**	**0.602**	**8**	**4.19**
4	**8**	**0.903**	**28**	**4.64**
5	**10**	**1.0**	**48**	**4.77**
6	**14**	**1.146**	**73**	**5.0**
7	**16**	**1.204**	**80**	**5.71**
8	**20**	**1.301**	**87**	**5.95**
9	**26**	**1.414**	**95**	**6.34**
10	**30**	**1.477**	**98**	**6.75**
11	**35**	**1.544**	**98**	**8.09**

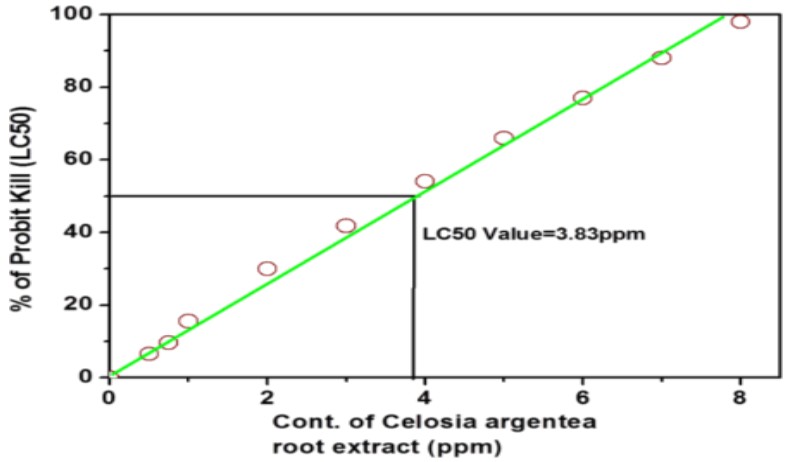

Fig. 2: Lethal concentration of *Celosia argentea* root extract against fourth instar larvae of *Aedesaegypti*

Table2: Determination of LC 50 *Celosia argentea*root extract

S. No	Conc. of *Celosia argentea* Root extract In ppm	Log Concentration	Percent of Kill (LC50)	Probit Kill Finney Table
1	**0**	**0**	**0**	**0**
2	**0.5**	**-0.301**	**6.5**	**3.87**
3	**0.75**	**-0.124**	**15.6**	**4.19**
4	**1**	**0.0**	**25.5**	**4.64**
5	**2**	**0.301**	**55**	**4.77**
6	**3**	**0.477**	**75**	**5.0**
7	**4**	**0.602**	**87**	**5.71**
8	**5**	**0.698**	**94**	**5.95**
9	**6**	**0.77**	**98**	**6.34**
10	**7**	**0.845**	**98**	**6.75**
11	**8**	**0.903**	**98**	**8.09**

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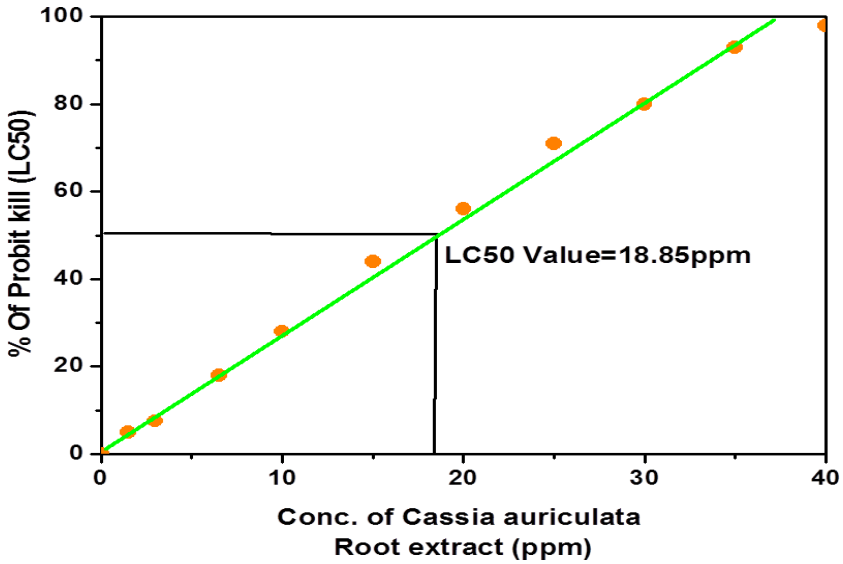

Fig. 3: Lethal concentration of whole plant extracts of *Celosia argentea* root extract against fourth instar larvae of *Aedesaegypti.*

C. Extraction and isolation of Celosin A from Celosia argentea root and leaf methanol extract

After 24 hours of maceration, dried leaves and roots (10 kg; *Celosia argentea*) were ground into a coarse powder and extracted with 50% aqueous ethanol at room temperature. The extract was concentrated under reduced pressure to give an ethanol extract (3.6 kg). A portion of the ethanol extract (3 kg) is chromatographed on D101 macroporous resin (10 kg) and washed alternately with water, 30%, 60% and 95% EtOH-water to obtain four fractions with yields 45.3, 50.7, 90. Acid hydrolysis of 18.9g of 3.

1 produced 2-hydroxy-23-carboxy-oleanolic acid and released monosaccharide units identified by common TLC and GC-MS as D-glucuronic acid (GlcA) and arabinose (Ara). sugar samples are tested together. The disaccharide nature of 1 was determined by the presence of two proton signals at 4.94 (d, J 7.8Hz) and 5.22 (d, J = 7.2Hz) in the 1H-NMR spectrum and assigned to units - glucuronic acid and - arabinose. , respectively. The H-H COSY experiment characterizes the fans and functions of proton resonances. Assignment to the carbon of the HMQC spectrum indicates that arabinose is terminal. The HMBC correlation between C-3 (82.27) and H-1 (4.19) of the GlcA unit and C-2 (84.03) of the GlcA unit and H-1 (5.22) of the Aha unit allowed the derivation of oleanolic acid Aha (1.2)-GlcA The structure of the disaccharide moiety at residue C-3. Based on the specific JH-1, H-2 coupling constant and 13C-NMR data, the relative stereochemistry of each monosaccharide was determined as -D-glucopyranose and -L-arabinopyranose. Thus, the structure of 2-hydroxy-23-aldehyde-3-O-[-L-arabinopyranosyl (1!2)-D-glucuronopyranosyl]-oleanolic acid is given as 1 as shown in Figure 5.

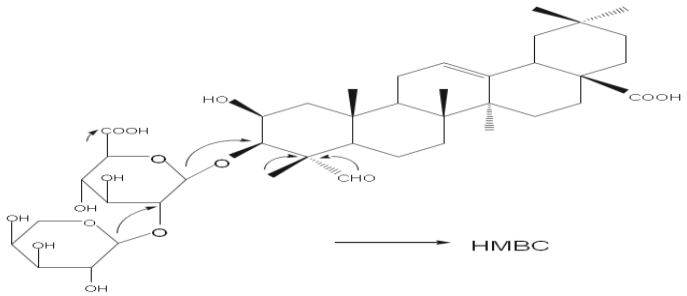

Fig. 4: HMBC Structure of Celosin A from the root and leaf

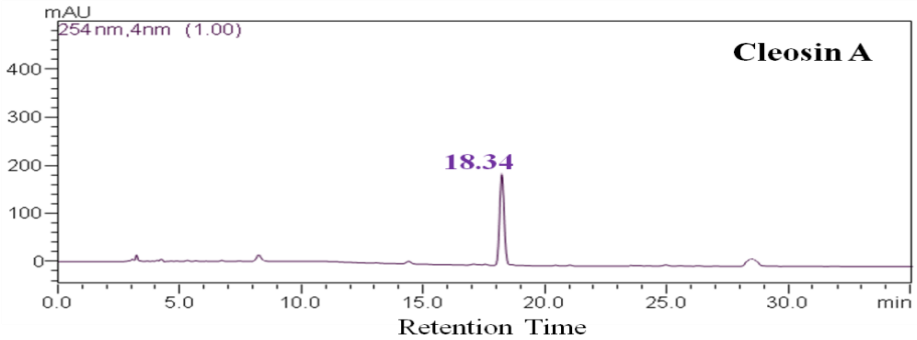

Fig. 5: HPLC analysis of Celosin A

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D. Larvicidal Bioassay of Celosin A isolated from Celosia argentea (leaf and root)

The bioassay testing Celosin A isolated from *Celosia argentea* (leaf and root) was tested at 0.25ppm/L to 6ppm/L. The entire larvae bioassay test with Celosin A showed a significant increase in mortality percentage with the increase of concentration. On comparing the tested flower extracts, maximum larvicidal activity was observed to be against early 4th instar larvae of *Aedesaegypti* with the LC50 were shown. The larvicidal bioassay test for *Aedesaegypti* recorded the LC50 in Fig. 5.The percent of probit kill was analysed base on the values of probit kill Finney table.

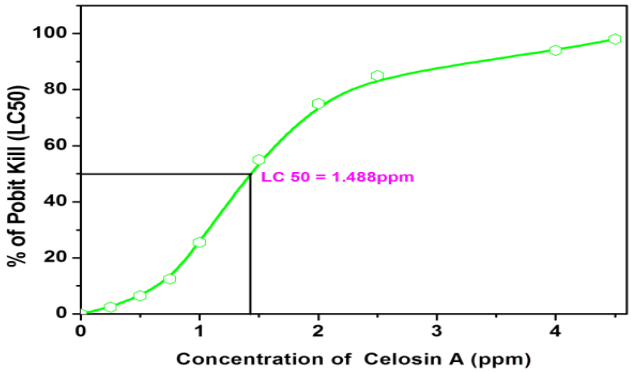

Fig.6: Lethal concentration of Celosin A of *Celosia argentea* against fourth instar larvae of *Aedesaegypti*.

IV. CONCLUSION

From the present study, extracts of Celosia argentea leaves and roots have proven to have a larvicidal effect. Raw extracts or isolated bioactive compounds from this plant (Celosin A, Emodin-1-O-Glycoside and Chrysophanol-1-O-Glycoside) can be used in stagnant waters where mosquitoes are known to breed. Analyzing, purifying and identifying the effective chemicals found in these species will lead to greater success in mosquito control. These extracts can be used for spraying in stagnant waters, which are known to be breeding grounds for mosquitoes, which are vectors of many diseases. Therefore, the large biomass of Celosia argentea, in southern India, particularly the states of Telangana and Andhra Pradesh, can be used as a biological resource for commercial production of mosquitoes.

DECLARATION

The authors declared that they have no conflicts of interest to this work.

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