



## Efficacy of *Phlogacanthus thyrsoformis* and *Clerodendrum glandulosum* Coleb. leaves and flowers crude extracts against malaria (*Anopheles stephensi*), dengue (*Aedes aegypti*) and filariasis (*Culex quinquefasciatus*) vector mosquitoes (Diptera: Culicidae)

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### Abstract

Mosquitoes are small irritating creatures that makes nuisance, their bites give itchy sensation, bumps and not only that but most importantly it transmit several vector borne diseases such as Malaria, Dengue, Chikungunya, Japanese encephalitis, filariasis etc. The mosquito vector control programme continues to rely on chemical insecticides, which causes resistance in vector species. Alternative methods based on phytochemical sources could be used to eliminate the Malaria, dengue, Chikungunya, and Zika virus vectors. In the present study we extracted *Phlogacanthus thyrsoformis* leaf and flower and the leaf of *Clerodendrum glandulosum* Coleb. with various solvents viz. petroleum ether, chloroform, ethyl-acetate and methanol against the 4<sup>th</sup> instar larvae of three species of mosquito, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. All the extracts at concentration of 25, 50, 100, 200 and 300 ppm caused varied level of mortality after 24 h of post treatment. Among the extracts methanol *Phlogacanthus thyrsoformis* flower extract showed the highest larvicidal activity with LC<sub>50</sub> and LC<sub>90</sub>= 36.157, 143.995 ppm for *Anopheles stephensi*; 55.088, 199.27 ppm for *Aedes aegypti*; 69.313, 267.125 ppm for *Culex quinquefasciatus*. The current study discovered that *Phlogacanthus thyrsoformis* methanol flower extract has remarkable larvicidal efficacy against medically important vector mosquitoes, making this plant product promising as an alternative to chemical insecticide in mosquito control programmes.

**Keywords:** *Phlogacanthus thyrsoformis*, *Clerodendrum glandulosum* coleb, crude extract, larvicidal, *anopheles Stephensi*, *aedes aegypti*, *Culex quinquefasciatus*

### Introduction

Mosquitoes are small and thoroughly studied insects globally. In tropical and sub-tropical region they are the causative agents for several mosquito-borne diseases such as Malaria, Dengue, Filariasis, Japanese encephalitis, Yellow fever, and newly Zika virus etc. [1]. Every year, approximately 500 million cases and 2.7 million deaths are reported as a result of mosquito-borne diseases [2]. Lymphatic filariasis is transmitted by the vector mosquito, *Culex quinquefasciatus*. The current global baseline of lymphatic filariasis patients is estimated to be more than 15 million people with lymphoedema and 25 million men with hydrocele. These chronic disease manifestations affect at least 36 million people [3]. Dengue fever is a vector-borne viral disease transmitted to humans by female *Aedes* mosquitoes. It is one of the world's most common diseases. Every year, nearly 390 million dengue viral infections occur, with 96 million developing clinical symptoms of varying condition of severe illness [4]. Infected female *Anopheles stephensi* bites transmit malaria disease to the people. There were an estimated 229 million malaria cases and 409000 malaria deaths in 2019 globally. Malaria affects children under the age of five the most, accounting for 67 percent (274 000) of all malaria deaths worldwide in 2019 [5]. One of the most effective ways of reducing these serious illnesses is to target the vectors and prevent disease transmission using synthetic insecticides. However, the use of synthetic chemical insecticides resulted in environmental

pollution and the development of physiological resistance in mosquitoes [6]. As a result, alternative, more effective, cost-effective, and environmentally friendly mosquito vector control agents, such as active ingredients of botanical source, must be sought. *Phlogacanthus thyrsoformis* and *Clerodendrum glandulosum* Coleb. are well known having a medically important bioactive compound as reported by the previous studies. Therefore, the current study was aimed to investigate the potential of *Phlogacanthus thyrsoformis* and *Clerodendrum glandulosum* Coleb. against three important species of mosquito vector larvae, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

*Phlogacanthus thyrsoformis* is an evergreen shrub in the *Acanthaceae* family. It grows in Northeast India, including Assam, Manipur, and Meghalaya, as well as parts of Bihar, North Bengal, the upper Gangetic plain, and bordering countries such as Bangladesh and Bhutan [7, 8]. Flowers have an antidote activity on pox and can help prevent skin diseases such as scabies and sores. In many communities, the flowers are eaten as vegetables. It also has antimicrobial properties and has been shown to be antibacterial, antifungal, anti-diabetic, anti-inflammatory, anti-cancerous, hypolipidaemic, and hepatoprotective [9]. In Meghalaya, the Jaintia tribe uses this plant because they believed it had historically significant antihyperglycemic, hypolipidemic, and hepatoprotective activity [10, 11, 12]. Anupam Kumar *et al.*, (2017) investigated the phytochemical constituents of *Phlogacanthus thyrsoformis*

leaf extracts, their antibacterial activity, and the cell cytotoxicity of silver nanoparticles derived from HeLa cells [13].

*Clerodendrum glandulosum* Coleb. belongs to the plant kingdom's *Verbenaceae* (Lamiaceae) family and It is a herb native to the North Eastern States of India [14]. The Apatani and Nyishi tribes of North-East India use CG leaves as a hypertension treatment [15, 16]. Jadeja R N *et al.*, (2011) reported that *Clerodendrum glandulosum* extract has strong hepatoprotective activity against CCl4-induced hepatic damage but has no toxic side effects [14]. Laboratory study has shown that *Clerodendrum glandulosum* leaf extract has antioxidant, lipid-lowering, and antihypertensive (personal communication) properties [17, 18].

## Materials and Methods

### Collection and preparation of crude extracts

Fresh matured different parts of *Phlogacanthus thyrsoformis* and *Clerodendrum glandulosum* Coleb. were collected from in and around the Bajengdoba, North Garo Hills (25°51'25.9"N 90°28'35.6"E), Meghalaya, India. Plants leaves and flowers were washed with tap water, shade dried and grounded into fine powder using electronic blender. The fine powder of different parts of each plant has extracted with various solvents *viz.*, petroleum ether, chloroform, ethyl-acetate and methanol using soxhlet apparatus. All the extracts like *Phlogacanthus thyrsoformis* petroleum ether leaf extract (PTPLE), *Phlogacanthus thyrsoformis* chloroform leaf extract (PTCLE), *Phlogacanthus thyrsoformis* ethyl-acetate leaf extract (PTELE), *Phlogacanthus thyrsoformis* methanol leaf extract (PTMLE), *Phlogacanthus thyrsoformis* petroleum ether flower extract (PTPFE), *Phlogacanthus thyrsoformis* chloroform flower leaf extract (PTCFE), *Phlogacanthus thyrsoformis* ethyl-acetate flower extract (PTEFE), *Phlogacanthus thyrsoformis* methanol flower extract (PTMFE), *Clerodendrum glandulosum* petroleum ether leaf extract (CGPLE), *Clerodendrum glandulosum* chloroform leaf extract (CGCLE), *Clerodendrum glandulosum* ethyl-acetate leaf extract (CGELE), *Clerodendrum glandulosum* methanol leaf extract (CGMLE) and were kept at -4°C in refrigerator for further work.

### Rearing of test organism

The test mosquito larvae of each species were collected from in and around Annamalai Nagar and reared in laboratory, Division of Vector Biology and Phytochemistry, Department of Zoology, Annamalai University, Annamalai Nagar. Larvae were feed (1ml per larva) with dog biscuits and yeast at 3:1 ratio. 10% sucrose solutions were provided to the adult mosquitoes and for female 1-week old chick were kept for blood meal (Govindarajan M *et al.*, 2012) [19]. Test mosquitoes were kept at 28±2°C, 70-85% relative humidity, with 14 h light and 10 h dark photo period.

## Bioassay test

### Larvicidal activity

Larvicidal test were carried out using WHO, (2005) standard protocol. Different concentrations (25, 50, 100, 200 and 300 ppm) of each extracts were prepared by adding appropriate dilution of plant extracts into disposable cup containing 100 ml of distilled water. 20 fourth instar larvae were introduced into the prepared concentration in the disposable cup and 5

replicates were made for each concentration. After 24 hour post treatment all the deaths larvae were counted and calculated the percentage mortality following the formula given below:

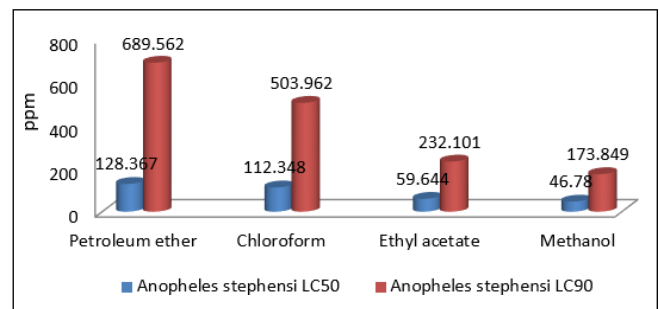
$$\% \text{ Mortality} = \frac{\text{Total No. of larval deaths}}{\text{Total No. of larvae introduced}} \times 100$$

### Statistical analysis

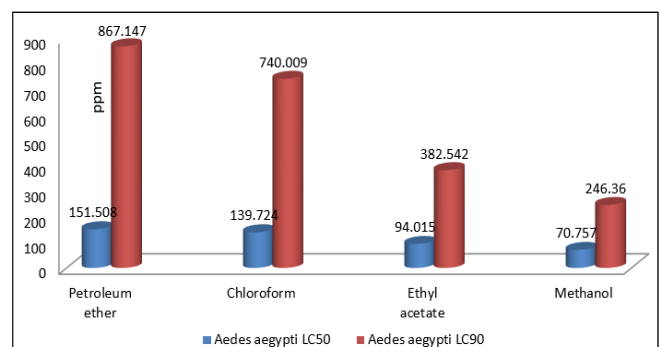
From five replicates the percentage mortality and standard error were calculated. All the data were subjected to probit analysis for calculating the LC<sub>50</sub>, LC<sub>90</sub> at 95% fiducial limit of upper confidence and lower confidence limit and chi-square values were calculated using IBM- SPSS Statistic version 22.0.

## Results

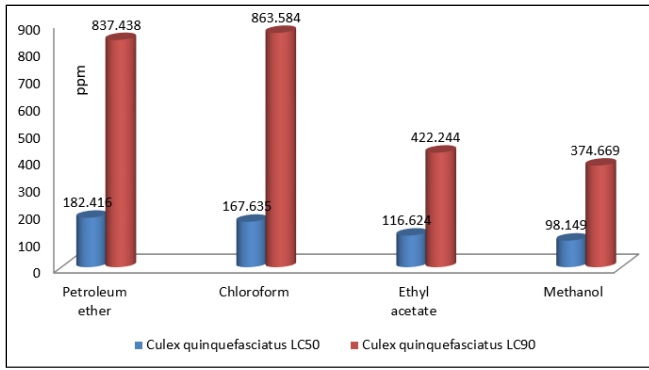
In the current study we carried out larvicidal effect of different crude extracts of *Phlogacanthus thyrsoformis* leaves and flowers *viz.*, PTPLE, PTCLE, PTELE, PTMLE, PTPFE, PTCFE, PTEFE, PTMFE and *Clerodendrum glandulosum* Coleb leaf extracts CGPLE, CGCLE, CGELE, CGMLE against the larvae of three different species of vector mosquitoes. All the crude extracts showed varied larvicidal activity after 24 hour of post treatment however, the highest activity was observed in PTMFE with LC<sub>50</sub> and LC<sub>90</sub> values of 36.157, 143.995 ppm for *Anopheles stephensi*; 55.088, 199.27 ppm for *Aedes aegypti*; 69.313, 267.125 ppm for *Culex quinquefasciatus*. All the results were shown in details in Fig.1 to Fig.3 for *Phlogacanthus thyrsoformis* leaf extracts, Fig. 4 to Fig.6 for flower extracts of *Phlogacanthus thyrsoformis* and *Clerodendrum glandulosum* Coleb leaf extracts were shown in Fig.7 to 9 against the *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae.



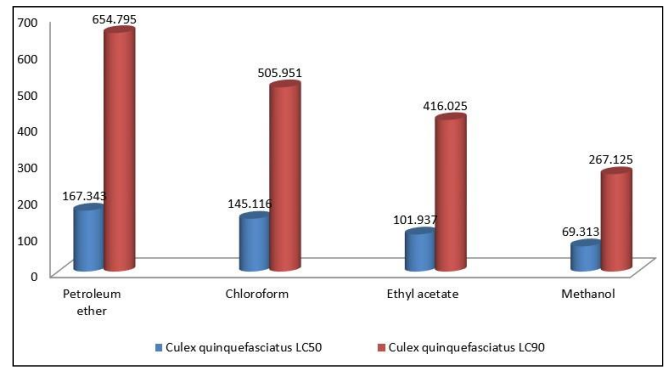
**Fig 1:** Larvicidal activity of *Phlogacanthus thyrsoformis* leaf extracts against the larvae of *Anopheles stephensi* for 24h of exposure time.



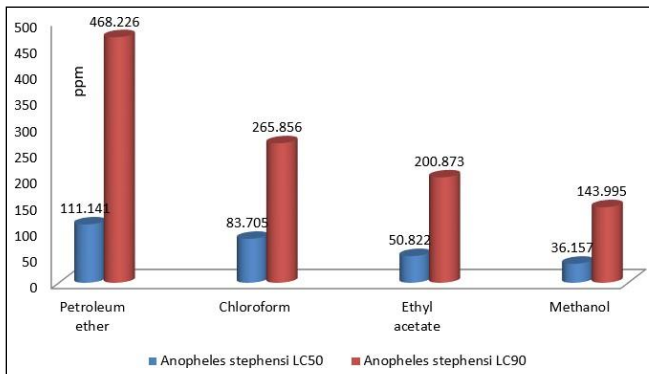
**Fig 2:** Larvicidal activity of *Phlogacanthus thyrsoformis* leaf extracts against *Aedes aegypti* larvae for 24h of exposure time.



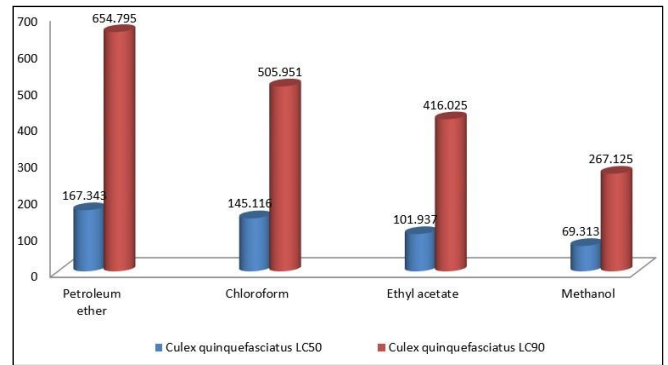
**Fig 3:** Larvicidal activity of *Phlogacanthus thyrsoformis* leaf extracts against *Culex quinquefasciatus* larvae for 24h of exposure time.



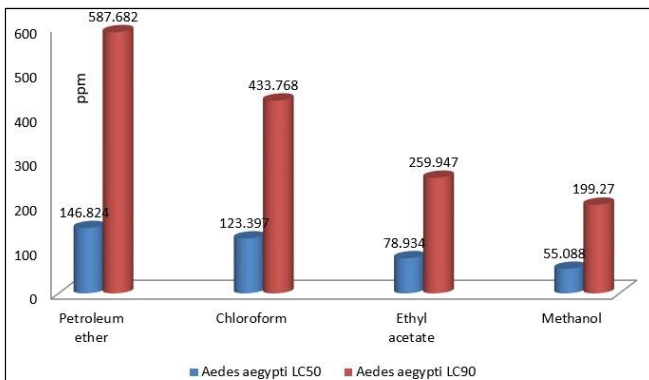
**Fig 7:** Larvicidal activity of *Clerodendrum glandulosum* leaf extracts against the larvae of *Anopheles stephensi* for 24h of exposure time.



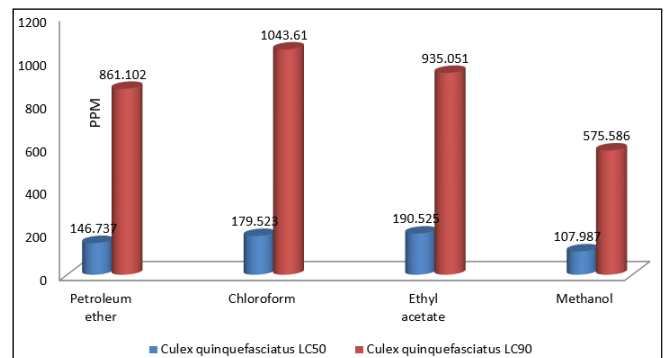
**Fig 4:** Larvicidal activity of *Phlogacanthus thyrsoformis* flower extracts against *Anopheles stephensi* larvae for 24h of exposure time.



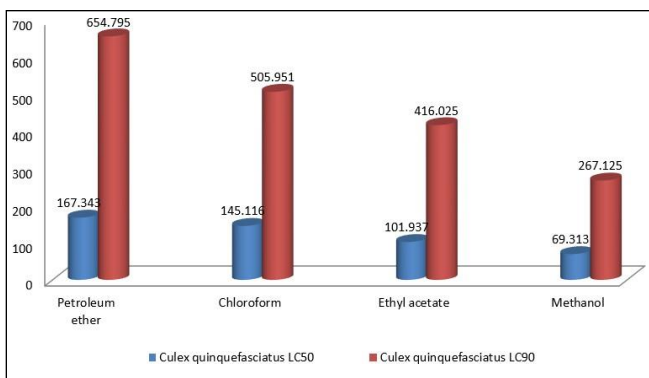
**Fig 8:** Larvicidal activity of *Clerodendrum glandulosum* leaf extracts against *Aedes aegypti* larvae for 24h of exposure time.



**Fig 5:** Larvicidal activity of *Phlogacanthus thyrsoformis* flower extracts against *Aedes aegypti* larvae for 24h of exposure time.



**Fig 9:** Larvicidal activity of *Clerodendrum glandulosum* leaf extracts against *Culex quinquefasciatus* larvae for 24h of exposure time.



**Fig 6:** Larvicidal activity of *Phlogacanthus thyrsoformis* flower extracts against the larvae of *Culex quinquefasciatus* for 24h of exposure time.

**Discussion**

Development of resistance to synthetic insecticides in vector mosquitoes, toxic problems, contamination of the environment and harmful effects of non-biodegradable debris on non-target creatures gives attention to search new methods and natural products alternative to synthetic insecticides to control vector mosquito [20]. To reduce the current rely on insecticide-based control of mosquito, bio-control approaches aim to be sustainable and target a variety of different mosquito species. Plants contain a wide variety of bio-active compounds that are similar to synthetic insecticides and may be an innovative, alternative to chemical insecticides in the future because they are safe for non-target life forms, humans, and are able to decompose [21]. Plant extracts may be more efficacious for controlling

resistant mosquitoes than specific bio-active compounds due to active ingredient synergisms [22, 23].

In the current study, *Phlogacanthus thyrsoformis* leaves and flowers and the leaves of *Clerodendrum glandulosum* were extracted with different solvents namely petroleum ether, chloroform, ethylacetate, and methanol and screened for their larvicidal activity against malarial vector, *Anopheles stephensi*, Dengue vector, *Aedes aegypti* and Japanese encephalitis vector, *Culex quinquefasciatus*. Among the extracts, the highest efficacy was recorded in (PTMFE) with LC<sub>50</sub> and LC<sub>90</sub> value of 36.157, 143.995 ppm for *Anopheles stephensi*; 55.088, 199.27 ppm for *Aedes aegypti*; 69.313, 267.125 ppm for *Culex quinquefasciatus*. Our results are comparable with the previous study of larvicidal efficacy of *Henbane* (*Hyoscyamus niger* L.) and *Oleander* (*Nerium oleander* L.) plants aerial parts extracts on *Anopheles* spp Larva which showed that *henbane* methanol flower extract has the highest larvicidal activity with LC<sub>50</sub> value = 0/26 ppm than leaves and stem extracts with [24]. Similar to our results Thangapandi Veni *et al.*, (2017) reported that the *Terminalia chebula* methanol extract showed higher activity on the larvae *An. stephensi* than *Ae. aegypti* and *Cx. quinquefasciatus* with the LC<sub>50</sub> values of 87.13, 93.24 and 111.98 ppm, respectively [20].

C. Kamaraj *et al.*, (2009) support our results as the highest larvicidal activity was observed in *C. auriculata* petroleum ether leaf and flower methanol extracts, *L. aspera* and *R. nasutus* methanol flower extracts, *S. torvum* methanol leaf and seed extracts and *V. negundo* hexane leaf extract against the *A. subpictus* larvae (LC<sub>50</sub>=44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm; LC<sub>90</sub> =187.31, 188.29, 233.18, 142.66, 151.60, 121.05 and 192.11 ppm, respectively) and against the *C. tritaeniorhynchus* larvae (LC<sub>50</sub>=69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm; LC<sub>90</sub> =335.26, 245.63, 300.45, 361.83, 185.09, 351.41 and 302.42 ppm, respectively) [25].

Similar to our results several previous study reports that increase in the concentration of plant crude extracts increase the mortality rate of the test organism and are the major cause of death rate, *Anopheles stephensi* larvae was more susceptible than *Aedes aegypti* and *Culex quinquefasciatus* larvae [26, 27]. Aylmarson N Sangma and K Vasudevan (2021) showed that the highest larvicidal activity was observed in methanol extract of *Prunus persica* and all the different extracts showed moderate activity after 24<sup>th</sup> hour of post treatment with LC<sub>50</sub> and LC<sub>90</sub> value 55.432, 221.027 ppm for *Anopheles stephensi*; 70.961, 321.561 ppm for *Aedes aegypti*; 104.104, 483.622 ppm for *Culex quinquefasciatus* [28].

*Senecio laetus* Edgew. methanol root extract has highest larvicidal activities among the other extracts tested after 24h of post treatment on vector mosquitoes with LC<sub>50</sub> and LC<sub>90</sub> value of 16.70 ppm, 109.19 ppm for *A. stephensi*; 22.30 ppm, 144.67 ppm for *Ae. aegypti*; 30.68 ppm and 183.84 ppm for *C. quinquefasciatus* [29]. *V. negundo*, *V. trifolia*, *V. peduncularis*, and *V. altissima* methanol leaf extracts were studied against *C. quinquefasciatus* 4th instar larvae with with LC<sub>50</sub> value of 212.57, 41.41, 76.28, and 128.04 ppm, respectively [30].

## Conclusion

The Present study showed that the *Phlogacanthus thyrsoformis* methanol flower extracts have promising larvicidal activities against the larvae of three mosquito

species and therefore, these crude extracts could be used as mosquitocide, alternative to synthetic chemical insecticide. The current study findings suggested that further research on the isolation of individual bioactive compound of *Phlogacanthus thyrsoformis* methanol flower extract on mosquito vectors is needed and it could be candidate as novel source of potential mosquitocide and could be use in vector control programmes. Therefore, Bioactivity-guided isolation of bioactive compound from *Phlogacanthus thyrsoformis* methanol flower extract on mosquito vectors is under progress that could have much value of commercial.

## Conflict of interest

Authors declare no conflict of interest.

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## References

1. Benelli G, Duggan MF. Management of arthropod vector data- Social and ecological dynamics facing the One Health perspective. *Acta Trop*,2018;182:80-91. Doi:10.1016/J.ACTATROPICA.2018.02.015
2. Bustamante, Jaleesa. "Mosquito Deaths & Mosquito Borne Disease Statistics [2020]." *MosquitoReviews*, 18 Dec, 2019. [mosquitoreviews.com/learn/disease-deathstatistic](https://mosquitoreviews.com/learn/disease-deathstatistic)
3. WHO Portal, <https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis>
4. Gou Y, Li Z, Fan R *et al.* Ethnobotanical survey and evaluation of traditional mosquito repellent plants of Dai people in Xishuangbanna, Yunnan Province, China. *J Ethnopharmacol*,2020;26:113-124. doi:10.1016/j.jep.2020.113124
5. WHO, 2019. <https://www.who.int/news-room/fact-sheets/detail/malaria>.
6. Abdalla M. Elimam, Khitma H. Elmalik, Faysal S. Ali. Efficacy of leaves extract of *Calotropis procera* Ait. (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes; *Saudi Journal of Biological Sciences*,211009:16:95-100
7. Nongthombam I, Das P, Devi J. Preliminary phytochemical screening of *Phlogacanthus thyrsoformis* Nees : A medicinal plant,2018;7(6):1156-1158.
8. K. Gitaranjan Singh, Sahana D., Dr. Rama T. and Kiran Kumar D.J. Phytochemical screening and antioxidant activity of methanolic and aqueous leaf extracts of *Phlogacanthus thyrsoformis*. *Int. J. Adv. Res*,2020;8(06):626-631
9. Singh SA, Singh NR. Antimicrobial. Activity of *Cassia didymobotrya* and *Phlogacanthus thyrsoformis*. *J. Chem. Pharm. Res*,2010;2(4):304-308.
10. Patwari B. A Glossary of Medicinal Plants of Assam and Meghalaya. 1st ed. Guwahati, India: MN Printers, 1992, 9.
11. Chakravarty S, Kalita JC. Antihyperglycaemic effect of flower of *Phlogacanthus thyrsoformis* nees on streptozotocin induced diabetic mice. *Asian Pac J Trop Biomed*,2012;2:S1357-61.
12. Kitlangki Suchiang, Nitasha Himanshu Kayde. Comparative phytochemical analysis of *Phlogacanthus*

- thyrsiflorus* nees: implications on attenuation of pro-oxidants and pathogen virulence in *Caenorhabditis elegans* model system; Asian Journal of pharmaceutical and clinical research, 2017, 10(5). DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i5.17319>
13. Anupam Kumar, Thounaojam Bidyapani, Digvijay Singh, Neeta Raj Sharma, Anand Mohan. Study of phytochemical compositions of leaves extracts of *Phlogacanthus thyrsiformis*, its antibacterial and silver nanoparticle derived cell cytotoxicity on HeLa cell line. *Journal of Pharmacy Research*, 2017, 11-12
  14. Ravirajsinh N Jadeja *et al.* Toxicological evaluation and hepatoprotective potential of *Clerodendron glandulosum*. Coleb leaf extract; *Human and Experimental Toxicology*, 2017;30(1):63-70.
  15. Kala CP. Ethnomedicinal botany of the Apatani in the eastern Himalayan region of India. *J Ethnobiol Ethnomed*, 2005;1:11.
  16. Deb S, Arunachalam A, Das AK. Indigenous knowledge of Nyishi tribes on traditional agroforestry systems. *Indian J Trad Knowledge*, 2009;8:41-46.
  17. Jadeja R N, Thounaojam M, Ansarullah, Ramachandran AV, Devkar R. Phytochemical constituents and free radical scavenging activity of *Clerodendron glandulosum*. coleb methanolic extract. *J Compl Integr Med*, 2009;6:19.
  18. Jadeja RN, Thounaojam MC, Ansarullah, Devkar RV, Ramachandran AV. *Clerodendrum glandulosum*. Coleb.- Verbenaceae, ameliorates high fat diet-induced alteration in lipid and cholesterol metabolism in rats. *Revista Brasileira de Farmacognosia*, 2010;20:117-123.
  19. Govindarajan M, Sivakumar R, Rajeswary M, Yogalakshmi K. Adulticidal activity of *Pithecellobium dulce* (Roxb.) Benth. against *Culex quinquefasciatus* (Say); *Asian Pacific Journal of Tropical Disease*, 2012;12:124-128
  20. Thangapandi Veni, Thambusamy Pushpanathan, Jeyaraj Mohanraj. Larvicidal and ovicidal activity of *Terminalia chebula* Retz. (Family: Combretaceae) medicinal plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*; *J Parasit Dis*, 2017;693-702.
  21. Benelli G, Lo-Iacono A, Canale A, Mehlhorn H. Mosquito vectors and the spread of cancer: an overlooked connection? *Parasitol. Res*, 2016;115:131-2137
  22. Vasudevan K, Malarmagal R, Charulatha H, Venkatalakshmi Saraswatula, Prabakaran K. Larvicidal effects of crude extracts of dried ripened fruits of *Piper nigrum* against *Culex quinquefasciatus* larval instars. *J. Vector Borne Dis*, 2009;46(1):153-156
  23. Maurya P, Sharma P, Mohan L, Verma MM, Srivastavav. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi*, *Asian Pac. J. Trop. Dis*, 2012;2:110-116.
  24. Mahmoodreza Behravan, Mohammad Reza Vaezi-Kakhki, Amin Baharshahi. Comparing larvicidal Effect of Methanol Extract of the Aerial Parts of Henbane (*Hyoscyamus niger* L.) and Oleander (*Nerium oleander* L.) plants on *Anopheles* spp Larvae (Diptera: Culicidae) *in Vitro*. *Zahedan J Res Med Sci*, 2017;19(3):e8631
  25. Kamaraj C, Bagavan A, Abdul Rahuman A, Abduz Zahir A, Elango G, Pandiyan G. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitol Res* 104, 2009:1163-1171 DOI 10.1007/s00436-008-1306-8
  26. Deepa M, Palanisamy K, Krishnappa K, Elumalai K. Mosquitocidal activity of *Polygala arvensis* Willd against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston.) and *Culex quinquefasciatus* (Say.) (Diptera: Culicidae). *Int. J. Mos. Res*, 2014;1:30-34.
  27. Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitology Research*, 2008b;103:691-695.
  28. Aylmarson N Sangma and K Vasudevan. Bio-larvicidal activity of *Prunus persica* (L) crude leaf extracts against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *International Journal of Entomology Research*, 2021;6(5):155-159
  29. Ali SI, Gopalakrishnan B, Venkatesalu V. Evaluation of larvicidal activity of *Senecio laetuis* Edgew. against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and Bancroftian filariasis vector, *Culex quinquefasciatus*. *South African Journal of Botany*, 2018;114:117-125
  30. Kannathasan K, Senthilkumar A, Chandrasekaran M, Venkatesalu V. Differential larvicidal efficacy of four species of *Vitex* against *Culex quinquefasciatus* larvae. *Parasitol Res*, 2007; 101(6):1721–1723
  31. Prasad A, Kumar A. Susceptibility status of dengue vector *Aedes aegypti* (L.) against various larvicides and insecticides in Udaipur district of Southern Rajasthan, India. *India. Int J Entomol Res*. 2019;5(1):74-7.