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LARVICIDAL ACTIVITY OF INDIGENOUS PLANT AQUEOUS EXTRACTS ON MALARIAL VECTOR, ANOPHELES SUBPICTUS GRASSI (DIPTERA: CULICIDAE)

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Abstract:

Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative bio control techniques in the future. The purpose of the present study is to assess the effect of leaf cold andHot water extracts of Ocimum basilicum,Ocimum americanum,Cleome gynandra and Passiflora foetida. All plant extracts showed moderate toxic effect on larvae after 24 h of exposure at 1,000 ppm;however, the highest larvicidal mortalityactivity was observed after 48 hcold water extracts of C.gynandra (LC_{50}=135.86, 155.58 and121.61ppm), (LC_{90}=594.12 720.87and 471.34 ppm) andP.foetida, (LC_{50}=140.60, 161.38 and126.72ppm), (LC_{90}=628.20 and 784.14–498.75ppm) and hot water extracts of O. americanum(LC_{50}=85.10, 89.40 and 67.19 ppm),(LC_{90} =364.12, 468.20–375.12ppm), and C.gynandra, (LC_{50}=167.82, 189.75and148.12ppm) (LC_{90} = 630.41, 782.11and492.94 ppm), and the overall findings of the present study have shown that the leaf aqueous plant extracts have the potential to be used as an ideal ecofriendly approach for the control of the A. subpictus. The screening results suggest that the cold water extract of C.gynandra, and hot water extract of O. americanumare promising in mosquito control.

Key Words:*Anopheles subpictus, plant extracts, cold and hot water, larvicidal activity*

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1 INTRODUCTION:

Malaria is one of the most common vector-borne diseases widespread in tropical and subtropical regions, including 64 parts of the America, Asia, and Africa (WHO 2007). Worldwide, there were about 247 million malaria cases with 0.881 million deaths reported in 2006 (WHO 2008). Malaria is the world's most dreadful tropical disease. Mosquito-borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2,100 million people at risk around the world (Kager 2002). As reported recently, 406 million Indians were at risk of stable *Plasmodium falciparum* transmission in 2007 with an uncertainty point estimate of 101.5 million clinical cases (95% CI 31.0–187.0 million cases; Hay et al. 2010). Rapid increases in population, limited funds, and know-how together with environmental change and an increase in the resistance of vectors and pathogens to insecticides and drugs and a shift in vector-control operations from long-term preventive measures to on-the-spot responses have led to an increase in vector-transmitted diseases (Gubler 1998). Malaria causes 1.3% loss in economic growth in Africa per year, and the long-term impact over a 15-year period is estimated at a 20% loss in the gross national product (Zaim and Guillet 2002). Thus, vector control is an important strategy in controlling and preventing vector-borne diseases such as malaria.

In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar et al. 2007). Malaria remains one of the most serious world health problems and the major cause of mortality and morbidity in the endemic regions. *Anopheles subpictus* is known to transmit malaria and filariasis in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local “frequent feeding strategy” in this primarily zoophagic and endophilic malaria vector (Amerasinghe and Amerasinghe, 1999). *Anopheles stephensi*transmits malaria in the plains of rural and urban areas of India. Malaria afflicts 36% of the world population i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions. In the South East Asian Region of WHO, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria and most of whom live in India. Of the 2.5 million reported cases in the South East Asia, India alone contributes about 70% of the total cases (Kondrachine, 1992).

The medicinal plant of *O. americanum L*., also known as hoary basil, lime basil, or American basil, is a medicinal and aromatic annual herb in the family Lamiaceae. It has a strong citrus smell, and the plant can grow between 15 and 35 cm in height with elliptic, pointy leaves and white or pale lilac flowers (Flora and Fauna 2023). Some species in this genus, such as *Cleome arabica* L, *Cleome visco* Linn, *Cleome droserifolia* (Forssk.), *Cleome* e nrichment,*Cleome rutidosperma* DC, *Cleome gynandra* L, possesses antipyretic and antidiarrheal properties, and have been used as traditional medicine in treatment of scabies, inflammation, rheumatic pains, blood problems, uterine complaints, malaria, counteract diabetic hyperglycemia, treat paralysis, anthelmintic problems, epilepsy, convulsions, spasm, pain, and skin disease(Motaal et al. 2011).The methanol extract of *Andrographis paniculata* was tested against fourth-instar larvae of *A. subpictus* and *C.tritaeniorhynchus* (Elango et al. 2009).The callus tissue of *Tagetes erecta* which showed the presence of insecticidal pyrethrin mixture was screened against *Tribolium* spp. Immediate ‘knock down’ effect was observed (Sarin, 2004),and the steam-distilled oils of *Tagetes patula*, *Tageteserecta* and *Tagetes minuta* were tested for larvicidal activity toward third instar *A. aegypti* and the results suggest a potential utilization of oil of *T. minuta* or its components for the control of *Aedes aegypti* and other species of mosquitoes (Green et al. 1991). The larvicidal potential of the essential oil of *T. patula* against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* was evaluated (Dharmagadda et al. 2005); the compounds, 4 thiophenes, 5-(but-3-ene-1-ynyl)-2,20 -bithiophene,5-(but-3-ene-1-ynyl)-50 -methyl-2,20 -bithiophene,2,20, 50, 200-terthiophene, and 5-methyl-2,20 ,50 ,200-terthiophene isolated from the floral extract of *T. minuta* were largely responsible for the toxicity exhibited against the adults of *A. aegypti* and *A. stephensi* (Perich et al. 1995).

As far as our literature survey could ascertain, no information was available on the larvicidal activity of the experimental plant species given here against A. *subpictus*. Therefore, the aim of this study was to investigate the mosquito larvicidal activity of aqueous extracts of four plant species from Tamil Nadu, India. The search for new strategies or natural products to control destructive insects and vectors of

diseases is desirable due to the prevalent occurrence of vector resistance to synthetic insecticides and the problem of toxic nonbiodegradable residues contaminating the environment and undesirable effects on nontarget organisms (Jantan et al., 2005). The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control.

2 Aim of the present study

The present study aims to evaluate the larvicidal activity of experimental plant aqueous extracts against *A. subpictus.*

3 MATERIALS AND METHODS:

3.1 Plant collection

The fully developed leaves of ***Ocimum basilicum,Ocimum americanum*** (Lamiaceae), *Cleome gynandra* (Cleomaceae), *Passiflora foetida* (Passifloraceae)(Fig 1), were selected on the basis of aromatic smell, bitter taste, resistance to damage by insect pests, ethnopharmacological, traditionally used medicinal value and ethnobotanical literature survey. The plant materials were collected from the Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13º13'4 N, 79º59'7E; Altitude 118 feet), Chennai, Tamil Nadu, and the taxonomic identification was made by Dr. A.Thalavai pandiyan, Department of Botany, Goverment Thirumagal Mills College Gudiyatham, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

3.2 Mosquito culture

A. subpictus larvae were collected from stagnant water area of Gudiyatham (12°56'41.05"N, 78°52'15.25"E) and identified in Zonal Entomological Research Centre, Vellore (12º552 .483"N, 79º724.83"E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out, at 27±2ºC and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively.

3.3 Preparation of plant extracts

The leaves were dried for 7-14 days in the shade at the environmental temperature (27-37ºC days time). The dried leaves (800 g) were powdered mechanically using commercial electrical stainless steel blender and extracted. For cold aqueous extracts, fresh parts of leaves were initially rinsed with distilled water and dried on a paper towel. The crude extracts were prepared by grinding the plant material in a mortar and pestle and passing the ground material through Whatman No 1 filter paper. Required concentrations of aqueous extracts were prepared by mixing the crude extract with a suitable amount of sterilized distilled water (Chowdhury et al. 2008). For hot water extract, the plant material was completely immersed in water in a round-bottomed flask, the water was brought to boiling, the distillate was collected, and required concentrations were prepared for the experimental test (Ross and Brian 1977). Finally, a series of filtrations to have an aqueous extract sterile. One gram of crude extract was first dissolved in 100 ml of acetone (stock solution). From the stock solution, 1,000 and 500 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. After this process the aqueous extract is ready to be used in bioassays of antilarvicidal activity.

The *A. subpictus* larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of Rahuman et al. (2000). For Bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with respective solvent and Polysorbate 80. The numbers of dead larvae were counted after 24 and 48 h of exposure, and the percentage mortality was reported from the average of five replicates. The experimental media, in which 100% mortality of larvae occurs alone, were selected for dose–response bioassay.

3.4 Dose–response bioassay

From the stock solution, different concentrations ranging from 31.25 to 1,000 ppm were prepared. Based on the preliminary screening results, crude different aqueous of leaf, extracts prepared from *O.basilicum,O. americanumC.gynandraP. foetida* were subjected to dose–response bioassay for larvicidal activity against *A. subpictus*. The numbers of dead larvae were counted after 24 and 48 h of exposure, and the percentage mortality was reported from the average of five replicates. However, at the end of 24 and 48 h, the selected test samples turned out to be equal in their toxic potential.

3.5 Statistical analysis

The average larval mortality data were subjected to profit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square

values were calculated by using the software developed by Reddy et al. (1992).

4 RESULTS AND DISCUSSION:

Mosquitoes serve as vectors of several diseases causing serious health problems to humans, and development of resistance towards chemical insecticides initiated a search for alternative control measures. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. The preliminary screening is a good means of evaluation of the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of cold water, and hot water, crude extracts of four plants are noted and presented in (Table 1 and Fig. 1a). All plant extracts showed moderate toxic effect on parasites after 24 h of exposure at 1,000 ppm;however, the 100% larvicidal mortalityactivity was observed after 48 h (Table 2 and Fig. 2). Cold water leaf extracts of *C.gynandra*(LC_{50}=135.86, 155.58 and121.61ppm), (LC_{90}=594.12 720.87and 471.34 ppm) and*P.foetida*, (LC_{50}=140.60, 161.38 and126.72ppm), (LC_{90}=628.20 and 784.14–498.75ppm) and Hot water leaf extracts of ***O.americanum***(LC_{90}=85.10,89.40 and 67.19ppm), (LC_{90} 364.12, 468.20 375.12ppm), and *C. gynandra*(LC_{90}=167.82,189.75and148.12 ppm) (LC_{90}=630.41,782.11and 492.94ppm) (Table 3 and Fig. 3), and The overall findings of the present study have shown that the leaf aqueous plant extracts have the potential to be used as an ideal ecofriendly approach for the control of the *A. subpictus*. The screening results suggest that the cold water extract of *C.gynandra*, and hot water extract of ***O.americanum***are promising in mosquito control against the larvae of *C. quinquefasciatus*, respectively.

The results are comparable with a earlier report, after 12 and 24 h of exposur larvicidal effects and mortality were observed in second- and third-instar larval mortality of *A. aegypti* increased after the treatment using *O. basilicum* and *O. americanum* essential oils at different concentrations (25–400 µg/mL), the treatment with *O. basilicum* at 25 g/mL caused 41.60% and 42.35% mortalities in the second- and third-instar larvae, and the values increased to 93.20% and 95.50% after a 24 h respectively, the LC_{50} of *O. basilicum* was the most effective against *A. aegypti* (LC_{50}: 37.14 and 31.43 ppm and LC_{90}: 286.55 and 233.43 ppm for the second and third instars, followed by that of *O. americanum* (LC_{50} 87.96 and 73.73 ppm LC_{90} 439.54 and 577.28 ppm) respectively, (Ganesan and Sornkanok 2023).

Earlier author reported that *O. americanum* can be regarded as the plant with the strongest larvicidal activity, in terms of potency and onset of action, against the larvae of *Ae. albopictus*. The hexane extract of the leaves of *O. americanum* was the only extract found to have larvicidal activity and mortality rates of 10.0 ± 0.0% and 23.4 ± 7.6% at 400 and 600 µg/mL respectively, Huimei et al. (2023). Several studies have evaluated the essential oils and solvent extracts derived from the leaves and/or stems of *O. americanum* for larvicidal activity against *Ae. aegypti* and reported LC_{50} values ranging from 15.03 µg/mL to 168 µg/mL for 24 h post-exposure (Chokechaijaroenporn et al. 1994; Prabhavathi et al. 2016).Bagavan et al. (2018) have reported that tested the crude extracts of *Citrus* peels against mosquito larvae and obtained the LC_{50} value of 58.25 ppm and LC_{90} value of 298.31 ppm with the chloroform extract of *Citrus sinensis* peels against *A.subpictus* larvae. Komalamisra et al. (2005) have reported that the petroleum ether (PE) and methanol (MeOH) extracts of *Rhinacanthusnasutus* and *Derris elliptica* exhibited larvicidal effects against *A. aegypti*, *C. quinquefasciatus*, *A. dirus*, and *Mansoniauniformis* with LC_{50} values between 3.9 and 11.5 mg/l, while the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/l. *D. elliptica* PE extract showed LC_{50} values of between 11.2 and 18.84 mg/l, and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/l. Kannathasan et al. (2008) have reported that the fatty acid methyl ester extract of *V. trifolia* showed the highest larvicidal activity with an LC_{50} value of 9.25 ppm followed by *V. altissima* (14.82 ppm) and *V. negundo* (18.64 ppm) against early fourth-instar larvae of *C. quinquefasciatus*. (Saravanan et al. 2007), the compounds 4-gingerol (1), (6)-dehydrogingerdione (2), and (6)-dihydrogingerdione (3) were isolated from petroleum ether extract of *Zingiber officinale* exhibited larvicidal activities against fourth-instar larvae of *A. aegypti* (LC_{50} 4.25, 9.80, 18.20 ppm) and *C. quinquefasciatus* (LC_{50} 5.52, 7.66, 27.24 ppm), respectively (Rahuman et al. 2008), The leaf extract of *Citrullus vulgaris* benzene, petroleum ether, ethyl acetate, and methanol were tested for larvicidial activity with LC_{50} values of 18.56, 48.51, 49.57, and 50.32 ppm, respectively, against *A.stephensi* (Mullai et al. 2008), larvicidal activity was found with the methanol extract of *V. trifolia* (LC_{50}=41.41 ppm) followed by *V. peduncularis* (LC_{50}=76.28 ppm), *V.altissima* (LC_{50}=128.04 ppm), and *V.negundo* (LC_{50}=212.57 ppm) on the early fourth-instar larvae of *C. quinquefasciatus* (Kannathasan et al. 2007). Rahuman and Venkatesan (2008) reported on the petroleum ether extract of *Citrullus colocynthis*,methanol extracts of *Coccinia indica*,

Cucumis sativus, *Momordica charantia*, and acetone extract of *Trichosanthesanguina* against the larvae of *A.aegypti* (LC50=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *C. quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. The hexane fraction of *Kaempferia galanga* was found to exhibit the highest larvicidal effect with the LC_{50} of 42.33 ppm against *C.quinquefasciatus* and possessed repellency against *C. tritaeniorhynchus* (Choochote et al. 1999). Extracts of *Achryrocline satureoides*, *Gnaphalium spicatum*, *Senecio brasiliensis*, *Trixisvauthieri*, *Tagetes patula*, and *Vernonia ammophila*were less active, killing more than 50% of the larvae only at the higher dose tested (100 mg/l) and the extract from *T. minuta and Eclipta paniculata* were the most active with a LC_{90} of 1.5 mg/l and LC_{50} of 1.0 mg/l and LC_{90} of 17.2 mg/l and LC_{50} of 3.3 mg/l against the larvae of *Aedes fluviatilis* (Macêdo et al. 1997).

Fig 1 Selected ethnobotanical medicinal plants

S.No	Binomial Name	Local Name	Photos
1	***Ocimum Basilicum***	**Thiruneetrupachilai**	
2	***Ocimum americanum***	**Kattuthulasi**	
3	***Cleome gynandra***	**Thaivelai**	

4	***Passiflora foetida***	**Siru poonaikali**	

Table 1 Larvicidal activity of aqueous crude extracts against early fourth-instar larvae of *A. subpictus* 24 hr at 1,000 ppm

Botanical name/family	Parts used	% Mortality (ppm)[a] ± SD	
		1	2
Cleome gynandra /(Cleomaceae)	Leaf	76±1.72	74±1.90
Ocimum basilicum/(Lamiaceae)	Leaf	54±1.00	66±1.52
Ocimum americanum /(Lamiaceae)	Leaf	57±1.56	79±1.98
Passiflora foetida /(Passifloraceae)	Leaf	80±1.98	69±1.11

Control-nil mortality
1Cold water 2 Hot water
a Mean value of five replicates

Fig 1a Lactivity of aqueous crude extracts against early fourth-instar larvae of *A. subpictus* 24hr at 1,000 ppm

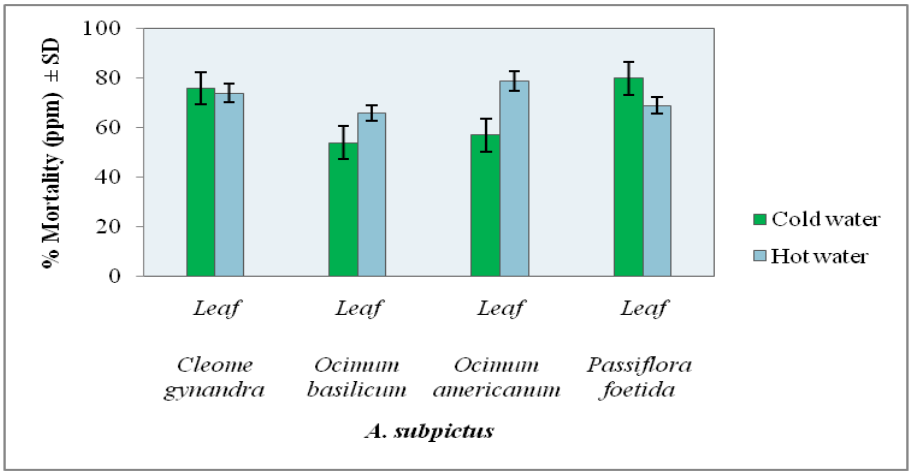

Table 2: Larvicidal activity of aqueous crude extracts against early fourth-instar larvae of *A.subpictus*48 hrat 1,000 ppm

Botanical name/family	Parts used	% Mortality (ppm)[a] ± SD	
		1	2
Cleome gynandra /Cleomaceae)	Leaf	100±0.00	100±0.00
Ocimum basilicum/(Lamiaceae)	Leaf	75±.156	71±1.72
Ocimum americanum /(Lamiaceae)	Leaf	69±1.98	100±0.00
Passiflora foetida /(Passifloraceae)	Leaf	100±0.00	87±0.00

Control—nil mortality
1cold water, 2 hot water
a Mean value of five replicates

Fig 2 Lactivity of aqueous crude extracts against early fourth-instar larvae of *A. subpictus*48 hrat 1,000 ppm

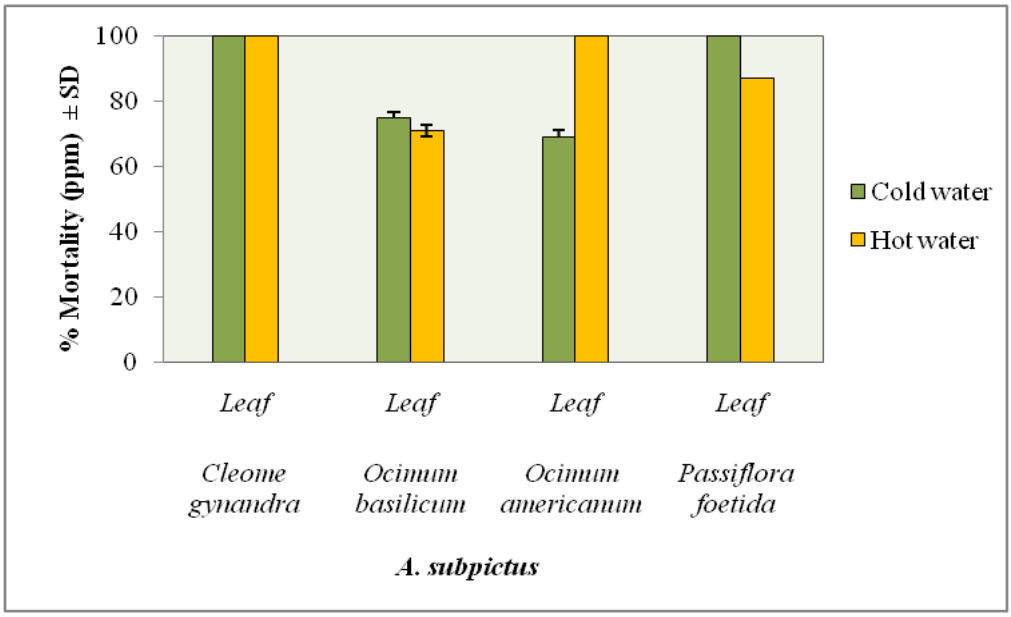

Table 3 Larvicidal activity of different solvent crude extracts against fourth-instar larvae of *A. subpictus*

Name of the plants	Solvents	LC_{50} ± SE (ppm)	(LCL-UCL)	LC_{90} ± SE (ppm)	(LCL–UCL)	$\chi 2$ (df=4)
C.gynandra	Cold water	135.86±8.16	(155.58-121.61)	594.12±62.12	(720.87–471.34)	9.96
	Hot water	167.82±12.11	(189.75-148.12)	630.41±78.14	(782.11–492.94)	8.66
O. americanum	Hot water	85.10.±10.06	(89.40-67.19)	364.12±70.70	(468.20–375.12)	12.8
P.foetida	Cold water	140.60±11.10	(161.38-126.72)	628.20±81.92	(784.14–498.75)	8.81

Control—nil mortality. Significant at P<0.05 level
LC_{50} lethal concentration that kills 50% of the exposed larvae, *LC_{90}* lethal concentration that kills 90% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, *$\chi 2$* chi-square; *df*degree of freedom

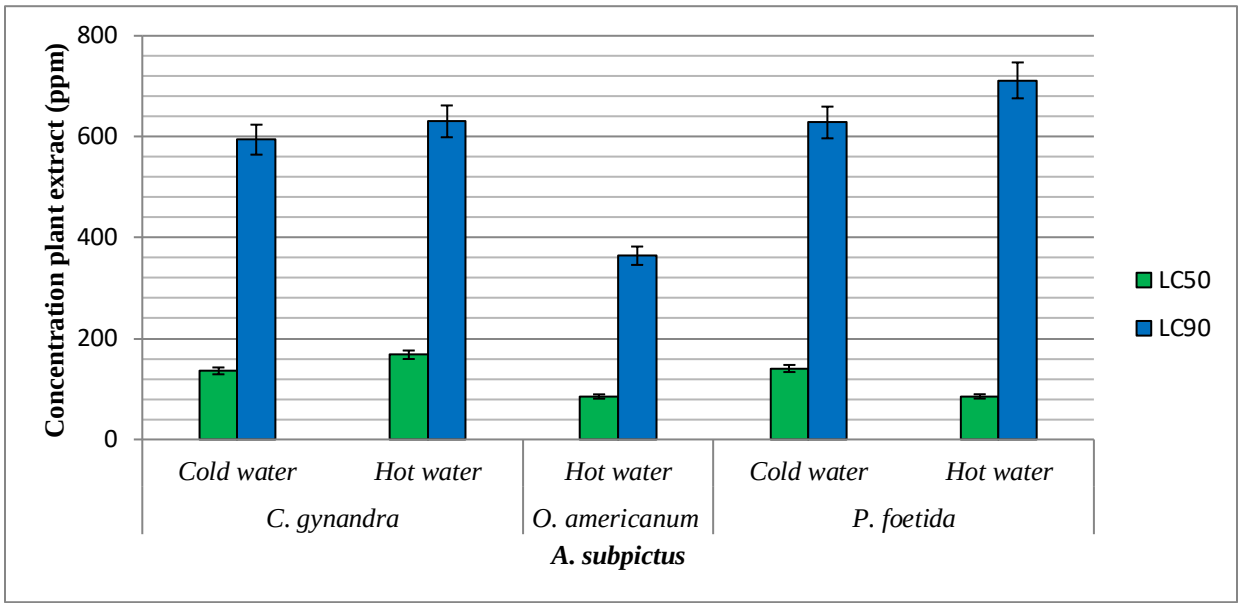

Figure 3 Larvicidal activity of different plant extracts against *A. subpictus* expressed as LC_{50} and LC_{90}

5 SUMMARY:

- Malaria is the world's most dreadful tropical disease. Mosquito-borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2,100 million people at risk around the world.
- In the present study, the plants were selected on the basis of the Indian entnophrmocolgical information and traditional uses provided by different literature sources.
- Four medicinal plants were collected from Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13º13'4 N, 79º59'7E; Altitude 118 feet), Chennai, Tamil Nadu, of South India.
- The experimental plants leaf was dried for 7-14 days in the shade at the environmental temperatures (27-37°Cday time). The dried leaf was powdered mechanically using commercial electrical stainless steel blender and extracted with cold and hot water.
- In the present study, larvicidal analysis and the *in vitro* activity against*A. subpictus*of leaf cold and hot water extracts of 4 medicinal plants.
- The outcome of this study strongly supports the development of new drug compound derived from plant products in the prevention against *A. subpictus*, which remains a devastating disease.
- The results of the study revealed that the *C.gynandra*,*O.americanum*and *P. foetida*showed a significant larvicidal effect with IC_{50} values of (LC_{50}=135.86±8.16,167.82±12.11, 85.10.±10.06), 140.60±11.10, µg/mL, respectively against *A. subpictus*.

6 CONCLUSIONS:

As far as our literature survey could ascertain, no information was available on the larvicidal activities of the experimental plant species given here against *A. subpictus*. Therefore, the aim of this study was to investigate the mosquito antilarvicidal activity aqueous extracts of four plant species from Tamil Nadu, India. Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friedly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. These effects may be due to the presence of neurotoxin compounds in plant extracts. No, behavioural changes were obtained in control group. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in vectors.

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