GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(Research Article)

Peels of *Citrus* fruits (*Citrus limon*, *Citrus hystrix*, and *Citrus sinensis*): A potent control agent against on dengue and chikungunya vector, Asian tiger mosquito *Aedes albopictus* L

Subramani Sudhakar [1], Ramasamy Prabhu [1], N. G. Tamil Selvi [2], S. Sridevi Subramanium [3], Subramanian Manjula [1], Arokiasamy Reni Prabha [1], and Jayapal Subramaniam [4, 5, 6, *]

[1] *Department of Zoology, Chikkaiah Naicker College, Erode-638 004, Tamil Nadu, India.*
[2] *Department of Zoology, Government Arts College, C. Mutlur, Chidambaram-608 102, Tamil Nadu, India.*
[3] *Department of Zoology, Arignar Anna Government Arts College, Sanyasikaradu (PO), Namakkal – 637 002, Tamilnadu, India.*
[4] *Division of Vector Biology and Control, Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.*
[5] *Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India.*
[6] *Division of Natural Products and Nanobiotechnology, Department of Zoology, Thiruvalluvar University, Serkkadu - 632 115, Tamil Nadu, India.*

GSC Advanced Research and Reviews, 2025, 22(02), 011–016

Publication history: Received on 11 December 2024; revised on 28 January 2025; accepted on 31 January 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.22.2.0026

Abstract

This study assessed the larval toxicity of peel extracts from *Citrus* fruits against the fourth instar larvae of the Asian tiger mosquito, *Aedes albopictus*, under laboratory conditions. The citrus peels were shade-dried at room temperature and coarsely powdered. *Citrus limon*, *Citrus hystrix*, and *Citrus sinensis* exhibit differing levels of larvicidal efficacy against various instars of *A. albopictus* larvae. The LC_{50} values for CLPE, CHPE, and CSPE against fourth instar larvae were 1.247 mg/mL, 1.235 mg/mL and 1.567 mg/mL, while the LC_{90} values were 2.807 mg/mL, 2.817 mg/mL and 3.291 mg/mL, respectively. The CLPE exhibits the highest larvicidal activity, followed by CHPE and CSPE. The current study demonstrates that the larvicidal potential of biowaste materials can be utilised as eco-friendly biopesticides. This approach may serve as an effective larvicidal agent and a low-cost vector control strategy, offering a viable solution to the ongoing dengue disease burden.

Keywords: *Aedes albopictus*; Vector Biology and Control; Biopesticides; *Citrus* fruits

1. Introduction

Mosquitoes impact human health and well-being primarily due to the transmission of mosquito-borne diseases and the annoyance caused by mosquito bites. Mosquitoes transmit malaria, dengue, and West Nile Virus, the biggest global concern. Although mostly found in tropical and subtropical regions, a major indigenous transmission cases of Chikungunya, dengue, rift valley fever, yellow fever, and Zika have been reported worldwide by the invasive Asian tiger mosquito (*Aedes albopictus*).

The World Health Organization have reported that, Dengue is more dangerous mosquito borne disease caused over 100 nations with the illness, with 70% of the global illness load in Asia. Dengue incidences peaked in 2023, affecting over 80

* Corresponding author: Jayapal Subramaniam

countries across all WHO regions. Since 2023, prolonged transmission and an unexpected rise in dengue infections have resulted to a record high of almost 6.5 million illnesses and 7,300 dengue-related deaths [1].

Vector-borne diseases, which account for 17% of all cosmopolitan infections, are regarded as significant global public health issues that impose a significant economic burden on the societies that are affected by these insidious pathogens. The emergence and re-emergence of these diseases in the last 40 years have been driven by population growth, urbanisation, globalisation, and poor public health infrastructure Abbasi et al., (2022); Wilson, et al., (2020).

Pathogen transmission prevention is essential to disease management to reduce illness-related mortality and economic losses. Vector population suppression may be a key chemical and non-chemical disease prevention breakthrough. For decades, insecticides were used to control mosquito larvae and adults, causing insecticide resistance in targeted insect populations. Vector resistance to the most common insecticides has been a major factor in the Vector Borne Disease elimination program's failure (Abbasi et al., 2022).

There is no vaccine or medicine for dengue fever, so controlling DHF requires eliminating *Aedes* mosquitoes. Since it still uses fogging to kill adult mosquitoes, the dengue eradication program has not been maximized. Fogging is expensive and requires special operations, limiting implementation. Eliminating larval and pupal sources will make prevention and eradication more sustainable. This requires an integrated mosquito control strategy using low-cost, safe, and environmentally friendly environmental, biological, and chemical methods. Due to this situation urgent need for alternative method to control of *Aedes* mosquitoes, by using natural resources (Moniharapon et al., 2020).

The *citrus* genus (Rutaceae) is a significant agricultural crop due to its nutritional compounds and valuable secondary metabolites. All components of citrus fruits, including the flesh, leaves, and rind, can be employed as food, spices, and medicinal agents. Certain citrus fruits, along with their peels and leaves, have been documented to exhibit bioactivities. *Citrus sinensis* exhibited antihyperlipidemic properties. *C. reticulata* peel has been reported to possess anti-aging properties for the skin. The fruit and peel of *C. limon* possess properties for the management and treatment of hypertension. *C. hystrix* exhibited antibacterial properties. *Citrus* fruits and leaves contain substantial amounts of polyphenols and flavonoids, including flavonols, flavones, flavanols, flavanones, isoflavones, and anthocyanidins such as hesperidin, tangeretin, rutin, nobiletin, and naringin (Gandhi et al., 2020). These compounds exhibit significant bioactivities as potent antioxidants and radical scavengers, as well as anti-inflammatory and anticancer properties. (Desmiaty et al., 2024)

Citrus plants are a significant category of horticultural commodities in India, prevalent across various regions of Southeast Asia. India hosts a diverse array of citrus varieties, and the citrus agro-industry generates substantial waste, comprising fruit peels, seed remnants, and pulp byproducts. These byproducts can constitute over 50% of the total weight of citrus fruits. *Citrus* peels are rich in phenolic compounds, flavonoids, and essential oils that exhibit bioactivity (Ma et al., 2009; Gorinsteina et al., 2001; Gomez-Mejía et al., 2019). In this present study, we attempt to formulate effective and safer mosquito control eco-friendly botanical insecticides from peel extracts of *Citrus* fruits (*Citrus limon*, *Citrus hystrix*, and *Citrus sinensis*) and evaluate the larvicidal activity against on dengue, zika, and chikungunya vector, *Aedes albopictus.*

2. Materials and method

2.1. Collection of plant materials

Fully matured peels of *Citrus* fruits (*C. limon*, *C. hystrix*, and *C. sinensis*) were collected from Naducombai and Karavalli villages in the Kollimalai Hills of Namakkal District, Tamil Nadu, India. It was verified by a plant taxonomist from the Department of Botany at Bharathiar University. A voucher specimen is archived at the Herbarium of the Division of Ecotoxicology, Department of Zoology, Chikkaiah Naicker College, Erode-4, Tamil Nadu.

2.2. Preparation and extraction crude extracts of Peels of *Citrus* fruits

Citrus fruit peels were rinsed with tap water and air-dried in the shade at ambient temperature. An electric blender powdered the desiccated plant materials (peels). From the powdered 300gm of plant material was extracted from the powder using 1 L of methanolic in a Soxhlet device for 8 hours (Vogel, 1978). The extracts were filtered using a Buchner funnel and Whatman number 1 filter paper. The crude plant extracts were subjected to evaporation until dry using a rotating vacuum evaporator. 1g of the plant residue was dissolved in 100 mL of acetone, resulting in a 1% stock solution. From this stock solution, several concentrations were generated at 0.50, 1.00, 1.50. 2.00 and 2.50 mg/mL, respectively.

2.3. Larvicidal bioassay test

A laboratory reared colony of *A. albopictus* larvae was used for the larvicidal activity. Twenty-five individuals of fourth instars larvae were kept in a 500 mL glass beaker containing 249 mL of dechlorinated water and 1-mL of the desired concentration of *Citrus* peels extracts (*Citrus limon*, *Citrus hystrix*, and *Citrus sinensis*) were added. Larval food was given for the test larvae. At each tested concentration, two to five trials were made and each trial consists of five replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae exposed to decholorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Mortality in treatment} - \text{Mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{\% of mortality} = \frac{\text{No. of dead larvae/pupae}}{\text{No. of larvae/pupae introduced}} \times 100$$

Figure 1 The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis (Finney, 1971)

2.4. Statistical analysis

Control mortality rates ranging from 1% to 20% were used to correct the observed mortality in our experiments, relying to Abbott's formula (Abbott, 1925). If control mortality rates were higher than 20%, experiments were discharged and repeated. Thus, probit analysis was used to calculate LC_{50} (LC_{90}) values and related parameters (Finney, 1971).

3. Results and Discussion

The larval mortality of *A. albopictus* following treatment with methanolic peel extracts of *Citrus* fruits, specifically *Citrus limon* peel extract (CLPE), *Citrus hystrix* peel extract (CHPE), and *Citrus sinensis* peel extract (CSPE), was observed. Table 1 presents the larval mortality results of *A. albopictus* IV instars following treatment at various concentrations (0.50 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL). Mortality rates of 31%, 29.20%, and 22.80% were observed in IV instar larvae treated with CLPE, CHPE, and CSPE, respectively, at a concentration of 0.50 mg/mL. These rates increased to 87.80%, 86.60%, and 77.80% for the same treatments at a concentration of 2.5 mg/mL. The LC_{50} and LC_{90} values were as follows: the LC_{50} value for the VI instar was 1.247 mg/mL for CLPE, 1.235 mg/mL for CHPE, and 1.567 mg/mL for CSPE. The LC_{90} values for the VI instar were 2.807 mg/mL for CLPE treatment, 2.817 mg/mL for CHPE treatment, and 3.291 mg/mL for CSPE treatment, respectively. In accordance with several previously reported plant-borne mosquitocidals and a dose-dependent effect was observed (Subramaniam et al. 2012a, b; 2015; 2016; 2017) and also acetogenins, alkaloids, alkamides, anthraquinones, coumarins, flavonoids, limonoids, polyacetylenes, sesquiterpene lactones, sterols, thiophenes, triterpenoids, and xanthones are the main chemical classes of bioactive plant extract marker compounds. Most of them are involved in inherent or induced plant chemical defence against microbes (fungi, bacteria, viruses) and hazardous insects (Pavela et al., 2019).

Plants serve as abundant sources of bioactive compounds suitable for the development of environmentally safe vector and pest management agents. Phytoextracts are being recognised as potential agents for mosquito control due to their low cost, ease of administration, and minimal risk. During the present study, three different citrus fruits peel extracts *C. limon*, *C. hystrix*, and *C. sinensis* were employed against larvae of *A. albopictus* and were exploited for their larvicidal potential against fourth instar larvae of *A. albopictus*.

Citrus peels are a rich source of polyphenolic compounds with potential health benefits, prompting numerous recent investigations on this theme (Ademosun et al., 2018). Citrus peels have transitioned from being considered waste to being utilised in laboratory settings. The peels are being investigated as raw materials, ingredients, or additives in the food, cosmetic, pharmaceutical, and nutraceutical industries (Rafiq et al., 2018). Several studies indicate that *citrus* peels are effective sources of bioactive compounds with significant antioxidant properties. The increasing focus on antioxidant research is associated with the relationship between degenerative diseases and oxidative damage (Oboh and Ademosun, 2012).

In tropical and subtropical regions, citrus fruits are widely consumed. Due to increased health awareness, citrus fruit consumption has increased worldwide. Last decade, global production reached 121 million tonnes (Patsalou et al., 2017). *Citrus* species are important in medicine and pharmaceuticals due to their nutritional content (Mabberley, 2004). The antioxidant activity of citrus metabolites protects and neutralises free radical-mediated disorders associated with diabetes (Boynes, 1991), cancer (Huang et al., 2001), neurodegenerative diseases (Perry et al., 2000), and cardiovascular diseases (Hool, 2006). This essay focusses on citrus secondary metabolites and their therapeutic effects. (Raghavan and Gurunathan, 2021).

In most of the medical practises, essential oils of various *Citrus* plants are prescribed as traditional medicines in all over the world as their active ingredients are responsible for numerous biological properties such as antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, anti-protozoal as well as insecticidal (Chouhan et al., 2019).

Natural products, such as plant-based products oils, crude extracts and phytochemicals serve as alternatives to synthetic pesticides owing to their efficacy and multifunctional characteristics, rendering them effective fumigants, insecticides, and repellents [Sousa et al., 2015]. Environmental and human health concerns related to chemical pesticides have prompted the exploration of natural compounds, such as plant essential oils, as alternative plant-based products for controlling *P. marginatus* insects [Isman et al., 2000, Isman et al., 2014]. Plant essential oil is a lipophilic compound capable of penetrating the wax layer and releasing toxic compounds into the insect body [Patil 2010]. Campolo et al. (2017) have reported that, the highest insecticidal activity of the citrus peel essential oils against the tomato borer *T. absoluta*. Similarly, El-Akhal et al. (2015) reported that *Citrus* oils exhibited significant larvicidal properties, achieving 100% mortality of *A. labranchiae* larvae at concentrations of 160 mg/L for *C. aurantium* and 640 mg/L for *C. sinensis*. The essential oil of *C. aurantium* exhibited the highest efficacy (LC_{50} = 22.64 mg/L, LC_{90} = 83.77 mg/L), whereas that of *C. sinensis* demonstrated the lowest efficacy (LC_{50} = 77.55 mg/L, LC_{90} = 351.36 mg/L).

4. Conclusion

The findings of the present investigation indicated that the novel fractions of crude extract derived from citrus fruit peels were highly effective in reducing the mosquito population, exhibiting 100% larvicidal activity at a minimal dosage. Extracts from *Citrus limon*, *Citrus hystrix*, and *Citrus sinensis* peels can be used as an organic alternative to synthetic chemical pesticides for vector control, thereby mitigating vector-borne diseases in an environmentally sustainable manner. CFPE (*Citrus* fruit peel extracts) did not cause harm to non-target organisms or the aquatic ecosystem.

Compliance with ethical standards

Acknowledgments

Dr. J. Subramaniam is grateful to the Science and Engineering Research Board (SERB), Department of Science and Technology (NPDF DST SERB/Project File No. PDF/2015/000650), New Delhi, India.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] WHO, 2024 World Health Organization, 2024 fact sheets |Dengue -severe Dengue https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue#:~

[2] Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, Lindsay SW. The importance of vector control for the control and elimination of vector-borne diseases, PLoS Neglected Trop. Dis. 14 (1) 2020. e0007831

[3] Abbasi E, Vahedi M, Bagheri M, Gholizadeh S, Alipour H, Djaefar Moemenbellah-Fard H. Monitoring of synthetic insecticides resistance and mechanisms among malaria vector mosquitoes in Iran: A systematic review. Heliyon 2022 8e08830

[4] Moniharapon DD, Ukratalo AM, Hendrajid Z, and Ramadhany MR. Biolarvicide of Herba Ethanol Extract of Phyllanthus niruri L on Aedes aegypti Mosquito Larva Vector Of Dengue Hemorrhagic Fever Disease (DHF). The 5th International Conference on Basic Sciences IOP Conf. Series: Journal of Physics: Conf. Series 2020 1463-012026; IOP Publishing doi:10.1088/1742-6596/1463/1/012026.

[5] Gandhi R, Gopalsamy AB, Vasconcelos S, Ding-Tao WU, Li HB, Antony PJ, Li H, Geng F, Gurgel RQ, Narain N, Gan RY. Citrus flavonoids as promising phytochemicals targeting diabetes and related complications: a systematic review of in vitro and in vivo studies. Nutrients 2020 12 (2907), 1–32. https://doi.org/ 10.3390/nu12102907.

[6] Desmiaty Y, Sandhiutami NMD, Mulatsari S, Maziyah FA, Rahmadhani K, Algifari HOZ, Jantuna FA. Antioxidant and anti-inflammatory activity through inhibition of NF-κB and sEH of some citrus peel and phytoconstituent characteristics. Saudi Pharmaceutical Journal 2024, 32, 101959.

[7] Ma YQ, Chen JC, Liu DH, Ye XQ. Simultaneous extraction of phenolic compounds of citrus peel extracts: effect of ultrasound. Ultrason. Sonochem. 2009 16 (1), 57–62. https://doi.org/10.1016/j.ultsonch.2008.04.012.

[8] Gorinsteina S, Martın-Bellosob O, Parkc YS. Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 2001 74 (3), 309–315.

[9] Gomez-Mejía, E, Rosales-Conrado N, Le´on-Gonz´alez ME, Madrid Y. Citrus peels waste as a source of value-added compounds: extraction and quantification of bioactive polyphenols. Food Chem. 2019, 295, 289–299. https://doi.org/ 10.1016/J.FOODCHEM.2019.05.136.

[10] Abbot WS. A method of computing of the effectiveness of an insecticide. J Econ Entomol 1925; 8: 265-267.

[11] Vogel AI (1978) Text book of practical organic chemistry. The English Language Book Society and Longman, London, p 1368

[12] Finney DJ, Probit analysis. Cambridge University Press, London, 1971 pp 68–72

[13] Subramaniam J, Kovendan K, Mahesh Kumar P, Murugan K, Walton W. Mosquito larvicidal activity of Aloe vera (Family: Liliaceae) leaf extract and Bacillus sphaericus, against Chikungunya vector, Aedes aegypti. Saudi J. Biol. Sci. 2012a, 19, 503–509. https://doi.org/10.1016/j.sjbs.2012.07.003

[14] Subramaniam J, Murugan K and Kovendan K Larvicidal and pupicidal efficacy of Momordica charantia leaf extract and bacterial insecticide, Bacillus thuringiensis against malarial vector, Anopheles stephensi Liston. (Diptera: Culicidae) Journal of Biopesticides, (2012b) 5, 301: 163-169.

[15] Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Mahesh Kumar P, Dinesh D, Chandramohan B, Suresh U, Nicoletti M, Higuchi A, Hwang JS, Kumar S, Alarfaj AA, Munusamy MA, Messing RH, Benelli G Eco-friendly control of malaria and arbovirus vectors using the mosquitofish Gambusia affinis and ultra-low dosages of Mimusops elengi-synthesized silver nanoparticles: towards an integrative approach?, Environmental Science and Pollution Research (2015). doi: 10.1007/s11356-015-5253-5.

[16] Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Dinesh D, Kumar PM, Chandramohan B, Suresh U, Rajaganesh R, Alsalhi MS, Devanesan S, Nicoletti M, Canale A, Benelli G. Multipurpose effectiveness of Couroupita guianensis-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with Aplocheilus lineatus predators. Environ. Sci. Pollut. Res. 2016 23, 7543–7558. https://doi.org/10.1007/s11356-015-6007-0.

[17] Subramaniam J, Murugan K, Jebanesan A, Pontheckan P, Dinesh D, Nicoletti M, Wei H, Higuchi A, Kumar S, Canale A and Benelli G. Do Chenopodium ambrosioides-Synthesized Silver Nanoparticles Impact Oryzias melastigma Predation against Aedes albopictus Larvae? Journal of Cluster Science 2017 28 (1), 413-436. (Impact Factor: 1.471).

[18] Pavela R, Maggi F, Iannarelli R, Benelli G. Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. Acta Tropica, 2019, Volume 193, Pages 236-271.

[19] Ademosun AO, Oboh G, Olasehinde TA, and Adeoyo OO. From folk medicine to functional food: A review on the bioactive components and pharmacological properties of citrus peels. Oriental Pharmacy and Experimental Medicine, 2018 18, 9–20.

[20] Oboh G, and Ademosun AO. Characterization of the antioxidant properties of phenolic extracts from some citrus peels. Journal of Food Science and Technology, 2012 49(6), 729–736.

[21] Patsalou M, Menikea KK, Makri E, Vasquez MI, Drouza C, Koutinas M. Development of a citrus peel-based biorefinery strategy for the production of succinic acid. J. Clean. Prod. 2017, 166, 706–716.

[22] Mabberley D, Citrus (Rutaceae): a review of recent advances in etymology, systematics and medical applications. Blumea-Biodiversity Evol. Biogeogr. Plants 2004 49 (2-3), 481–498.

[23] Boynes J. Role of oxidative stress in development of complications in diabetes. Diabetes 1991, 40, 405–411.

[24] Huang RP, Peng A, Golard A, Hossain MZ, Huang R, Liu YG, Boynton AL. Hydrogen peroxide promotes transformation of rat liver non-neoplastic epithelial cells through activation of epidermal growth factor receptor. Mol.: Publ. Cooperation Univ. Texas MD Anderson Cancer Center, 2001, 30 (4), 209–217.

[25] Perry G, Raina AK, Nunomura A, Wataya T, Sayre LM, Smith MA. How important is oxidative damage? Lessons from alzheimer's disease. Free Radic. Biol. Med. 2000 28 (5), 831–834.

[26] Hool LC. Reactive oxygen species in cardiac signalling: from mitochondria to plasma membrane ion channels. Clin. Exp. Pharmacol. Physiol. 2006 33 (1-2), 146–151.

[27] Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, and Nayik GA. Citrus peel as a source of functional ingredient: A review. Journal of Saudi Society of Agricultural Sciences, 2018 17, 351–358

[28] Raghavan S and Gurunathan J. Citrus species – a golden treasure box of metabolites that is beneficial against disorders Author links open overlay panel Journal of Herbal Medicine, 2021 Volume 28, , 100438

[29] Chouhan KBS, Tandey R, Kumar Sen K, Mehta R, Mandal V. Critical analysis of microwave hydrodiffusion and gravity as a green tool for extraction of essential oils: Time to replace traditional distillation. Trends in Food Science & Technology, 2019 Vol. 92, P. 12-21.

[30] Sousa RMOF, Rosa JS, Oliveira L, Cunha A, Fernandes-Ferreira M. Activities of Apiaceae essential oils and volatile compounds on hatchability, development, reproduction and nutrition of Pseudaletia unipuncta (Lepidoptera: Noctuidae), Ind. Crop. 2015 Prod. 63 226–237, https://doi.org/10.1016/j. indcrop.2014.09.052.

[31] Isman MB, Plant essential oils for pest and disease management, Crop Protect. 19 (2000) 603–608, https://doi.org/10.1016/S0261-2194(00)00079-X.

[32] Isman MB, Grieneisen ML. Botanical insecticide research: many publications, limited useful data, Trends Plant Sci. 2014 19 140–145, https://doi.org/10.1016/j.tplants.2013.11.005.

[33] Patil SV, Salunke BK, Patil CD, Salunkhe RB, Gavit P, Maheshwari VL. Potential of extracts of the tropical plant Balanites aegyptiaca (L) Del. (Balanitaceae) to control the mealy bug, Maconellicoccus hirsutus (Homoptera: Pseudococcidae), Crop Protect. 2010 29 1293–1296, https://doi.org/10.1016/ j.cropro.2010.05.016.

[34] Campolo O, Cherif A, Ricupero M. Citrus peel essential oil nanoformulations to control the tomato borer, tutaabsoluta: Chemical properties and biological activity. Sci. Rep. 2017;7: 13036.

[35] El-Akhal F, Abdelhakim El Ouali Lalami, Raja Guemmouh (2015). Larvicidal activity of essential oils of Citrus sinensis and Citrus aurantium (Rutaceae) cultivated in Morocco against the malaria vector Anopheles labranchiae (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease 2015 Volume 5, Issue 6, Pages 458-462.