PARADE TOWARDS GREEN REVOLUTION: LARVICIDAL ACTIVITY OF CATUNAREGAM SPINOSA AGAINST AEDES AEGYPTI

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Abstract

Dengue is a common vector-borne disease prominent in tropical and subtropical climates. It causes losing of millions of human lives per year around the world. *Catunaregam spinosa* is an underrated medicinal plant in Sri Lanka which rumored in possessing larvicidal activity against mosquitoes. Present study investigated the toxicity of seed extract of *C. spinosa* against fourth instar larvae of *Aedes aegypti* L to provide a scientific validation to the embedded property and to support the ethnobotanical vector control approaches. Mosquito larval cultures exposed to a series of concentrations (75.0, 125.0, 250.0, 500.0, 1000.0 mg L^{-1}) showed concentration dependent mortalities and teratogenic effects after 24 hours. Statistical analysis computed 24 h, LC_{50} as 233.67 mg L^{-1} and LC_{90} as 659.93 mg L^{-1} reporting a moderate larvicidal activity. Preliminary phytochemical analysis revealed the presence of alkaloids, coumarins, saponins and flavonoids. Presence of butanoic acid, octadecanoic acid, n-hexadecenoic acid and palmitic acid along with 19 compounds were identified using Gas Chromatography Mass Spectrometry. In conclusion, the study unveils a lodged property in an abandoned plant in Sri Lanka whilst supporting the green-revolution and sustainable health system for future developments of bio-larvicides using natural compounds available in *C. spinosa*.
Keywords: *Aedes aegypti*, bioinsecticide, *Catunaregam spinosa*, green revolution, vector control

Introduction

Mosquito diseases spreading via parasites, bacteria or viruses account for more than 700,000 worldwide annual deaths. Malaria (parasite) and dengue (virus) transmitted by *Anopheles* and *Aedes* mosquitoes are responsible for more than 3.4 million confirmed cases and 40,000 deaths in every year [1]. Sri Lanka as a tropical country provides preferable conditions for rapid life cycles completion of *Aedes aegypti* L. Number of cases of dengue has consistently increased in Sri Lanka since start of 2023 which is three times greater than that of corresponding period in 2021 and 2022 [2].

Life Cycle of *Ae. Aegypti*

Chikungunya, zika virus, yellow fever, Japanese encephalitis, dengue and dengue hemorrhagic fever are transmitted by mosquitoes belongs to genus *Aedes*. *Aedes aegypti* mosquito life cycle is consisted of four developmental stages consisting eggs, larvae, pupae and adults (Figure 1).

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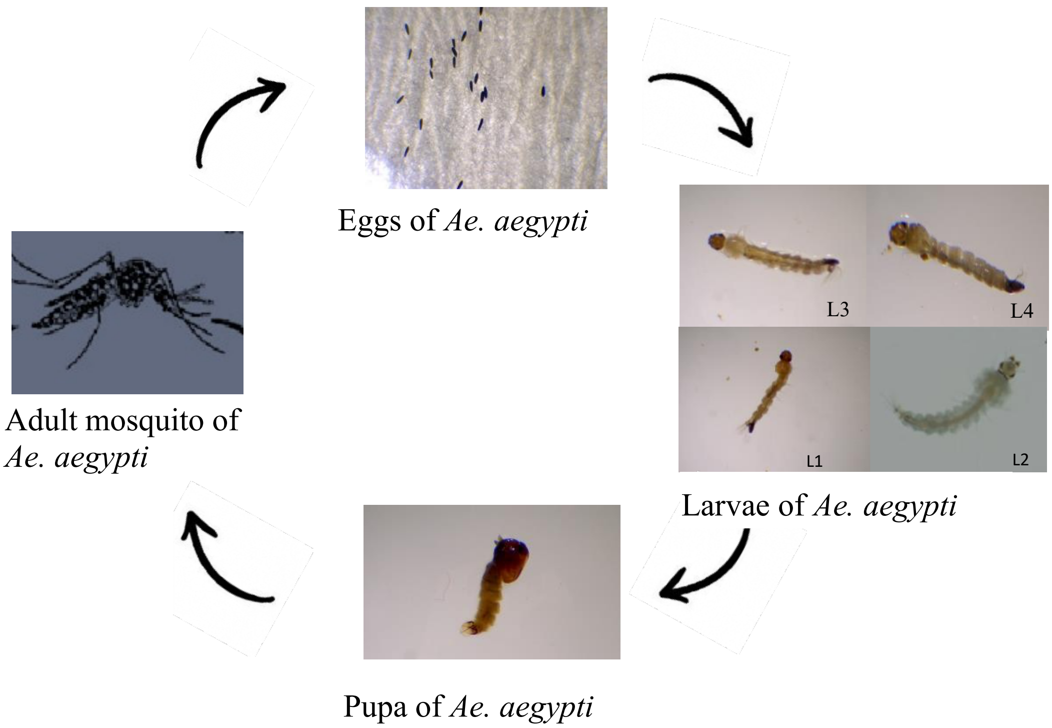

Figure 1: Development life cycle of *Ae. aegypti*

Mosquitoes of *Ae. aegypti* lay about 200 eggs per oviposition and only takes 8 days to complete the life cycle. First two larval stages are formed after first two days of egg hatching and the third instar remains in the next two days.

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Vector Control Approaches

Over the past decades mosquito vector control approaches were linked mostly with chemical, and physical attributes where the current traits are articulated with molecular and ethnobotanical approaches.

Chemical, physical and molecular approaches

There are several principal interventions in vector control approaches where the mosquito management is primitively composed of eliminating sources and the respective vectors of spreading the disease. The approaches are attributed with chemical, physical, molecular and ethnobotanical perspectives. Chemical perspectives such as insecticides, larvicides and non-chemical perspectives including mass trapping of larvae, oil coating, use of larvivores fish, habitat management, suppressing the population via genetically engineered techniques involve in vector control approaches.

Use of mosquito repellents, insecticide treated mosquito nets, covering arms and legs with long clothes, using nets to screen mosquitos at the open doors and windows are common physical preventive measurements in the current society of Sri Lanka.

Deficiency and the absence of vaccines trigger the inauguration of new vector control strategies via investigating different biological aspects to combat the surging mosquito population. Studies have been conducted for reducing the mosquito population by employing genetically engineered male mosquitos where the introduced fatal genes are transmitted to offsprings causing them to die before getting sexually matured. For an instance, Molecular

Medicine Unit of Faculty of Medicine, University of Kelaniya, Sri Lanka conducted a mass project of releasing 100,000 of sterile male mosquitos which made sterile by Sterile Insect Technique (SIT) to a dengue abundant area with the aim of reducing offsprings by suppressing the mating and oviposition frequency of adults [3]. It revealed remarkable reduction of mosquito population at the end of the study period.

Further researches mentioned the use of dispensers that emit neurotransmitters such as neuropeptide Y less attracts the mosquitos to humans. Developing nano technology integrated insecticides are an evolving concept where fungi, bacteria, algae or plant extracts are used as carriers to reduce and stabilize the preferred compounds into nanoparticles [4]. Balaraman et al. (2022) used aqueous extracts of *Sargassum myriocystum* to synthesize Titanium Dioxide nanoparticles (TiO_2-NPs) against *Ae. aegypti* and *Culex quinquefasciatus* [5]. Manojkumar et al. (2023) estimated the LC_{50} and LC_{90} of Zinc Oxide nanoparticles (ZnO-NPs) developed using *Brassica oleracea* var. botrytis as 76.03, 190.03 ppm which tested against the fourth instar of *C. quinquefasciatus* mosquito larvae [6].

Ethnobotanical approaches

High cost, advanced technologies, harmful environmental impacts and resistance build up from insects decelerate the demand for chemical insecticides where additional strict rules and regulations upon synthetic insecticides arouse the interest on botanical insect management strategies. Application of ethnobotanicals is a raising technique in controlling mosquitoes as of the easy degradability, less persistence in the environment, less harmfulness to vulnerable parties and the cost effectiveness. Bio-insecticides are usually derived from bio active compounds extracted from plant materials or microorganisms [7]. Entamopathegenic fungi orders such as Entamophthorales, Hypocreales and predatory fish genera such as *Gambusia*, *Poecilia* and copepods are used in managing mosquito populations [8,9].

Diverse phytochemical compositions found over 400,000 plant species unlocks the pathways in developing eco - friendly insecticides. Leaves, fruits, root, stem bark and seeds belong to different plant families such as Fabaceae, Piperaceae, Asteraceae, Apocynaceae and Euphorbaceae possess significant toxicity against *Ae. aegypti* [10-13]. Kumar et al. (2012) studied toxicity of 15 local plants grown in New Delhi against larvae of *Ae. aegypti* and revealed 10 species as potent sources where the *Achyranthes aspera*, *Zingiber officinalis*, *Ricinus communis*, *Trachyspermum ammi* and *Cassia occidentalis* exhibited significant activity as the Median Lethal Concentration (LC_{50}) ranged from 55.0 to 74.67 mg L^{-1} [14]. Organophosphates, carbomates and pyrethroids are historically used synthetic larvicides and essential oils, terpenoids, phenyl propanoids, thiophenes, alkaloids, tannins, coumarins, geraniol are naturally occurring mosquitocidal compounds [15]. Plants belong to family Rubiaceae show larvicidal activity against *Ae. aegypti* at different concentrations. Methanolic and acetone leaf extracts of *Gardenia ternifolia* found in Kenya exhibited 32.01 mg L^{-1} and 83.31 mg L^{-1} of LC_{50} respectively [16]. Carvalho et al. (2014) studied larvicidal activity of *Spermacoce latifolia* found in Brazil and revealed LC_{50} of acetone, methanol and n-Hexane extracts as 574.0, 625.0 and 415.0 mg L^{-1} respectively [17].

Catunaregam spinosa

Catunaregam spinosa is an underrated medicinal plant in Sri Lanka which belongs to family Rubiaceae. *C. spinosa* grows up to 1-10 m as a large deciduous thorny shrub or small tree (Figure 2a). Its shiny leaves are arranged in groups of three and are 0.75-3.5 inches in length and 0.4-1.2 inches in width (Figure 2b). White, pale yellow flowers of *C. spinosa* emit a honey-like fragrance and bloom at the terminals of the lateral branches, either singly or in clusters (Figure 2c). The berry-like fruits are small, measuring 2-6 inches, with a hard pericarp and fleshy interior (Figure 2d). Seeds are smooth, compressed, oval shaped, and embedded in a dark, fetid pulp with a prismatic shape (Figure 2e).

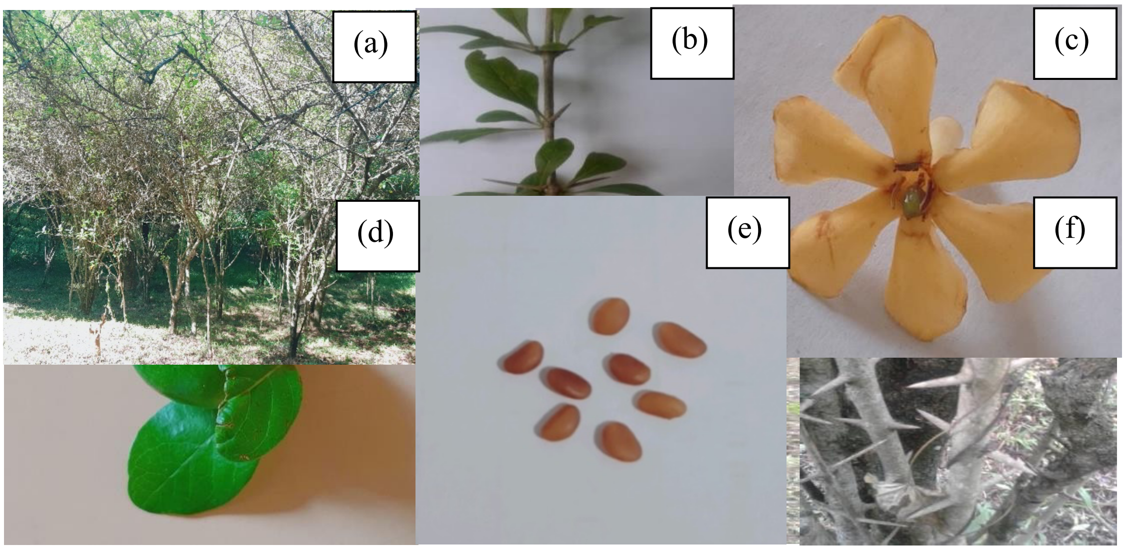

Figure 2: (a) habitat (b) leaves (c) flower (d) fruit (e) seeds (f) spines of *C. spinosa*

The plant possesses crucial pharmacological properties of anti-inflammatory, anti-oxidant, cytotoxicity, anti-bacterial, insecticidal, piscicidal, anti-microbial and anthelmintic in different parts including leaves, fruits, stem bark, roots and seeds [18-22]. Glycosides, triterpenoids, saponins, flavonoids and alkaloids are major secondary metabolites found in *C. spinosa* which are responsible for mentioned crucial properties [23,24].

Global and Sri Lankan Distribution

C. spinosa is distributed among several tropical and sub-tropical countries (Bangladesh, Sri Lanka, Indonesia, China, Malaysia, Vietnam, most of the jungles in India and in African countries such as Tanzania, states of Texas, California and Arizona in United States, Middle East countries) [25] where the same regions are high prevalence of dengue. According to the "Flora of Ceylon", *C. spinosa* employs either as a single plant or small to large clusters in most of the districts in Sri Lanka; Jaffna and Mannar – northern region, Puttalam, Polonnaruwa and Anuradhapura – north central region, Trincomalee and Batticaloa – north eastern region, Matale, Kandy, Badulla, Haldumulla and Rathnapura – central region, Hambantota and Monaragala – south eastern region, Nawinna – south western region [26].

Investigating the larvicidal activity of abundant *C. spinosa* is worth and timely as insecticidal, larvicidal and mosquito repellent activities are less concerned and poorly studied both in worldwide and nationwide context [27-29]. Aboriginal people lived in different localities of the world used *C. spinosa* to repel mosquitoes. Pawar et al. (2008) mentioned applying extracts of fruits on skin by people who lived in Jalgoan District in India [30]. Navinkumar et al., (2019) mentioned employing crushed parts of the plant in paddy fields to repel insects using the strong smell disliked by insects with no proven laboratory studies [31]. Wuillda et al. (2019) reviewed larvicidal activity of secondary metabolites found in 85 plant species against *Ae. aegypti* from the data collected from literatures during 2013 to 2018 where no data was found occupying of *C. spinosa* [32]. Anoopkumar et al. (2020) studied larvicidal activity of petroleum ether, ethanol, acetone and aqueous seeds extracts of *C. spinosa* grown in India against *Ae. aegypti* as the only laboratory experiment over the desired activity [33]. Geological and ecological variations such as rainfall, soil type and temperature play key roles in determining phyto- potencies of plants as of leading different phytochemical profiles and strengths. The variations cause for different lethal concentrations resulting available for unique regional vector control approaches. Therefore, the present study will provide details about larvicidal activity of *C. spinosa* grown in Sri Lanka which will facilitate in extracting the potential of this under-utilized plant and mitigating the dengue virus spreading, co-currently supporting the global trend of green revolution. Also, the titled study used larvae as the test organism to improve the efficacy of using the property before mosquitoes completing their life cycle.

Methodology

Larvicidal activity of *C. spinosa* grown in Sri Lanka was investigated establishing suitable mosquito cultures, collecting authenticated plant materials, extracting and analyzing the phytochemicals and conducting the assay according to the guidelines by WHO.

Establishment of mosquito culture

Initial *Ae. aegypti* mosquito colony was established using eggs collected from wild female mosquitoes caught from Medical Officer of Health area, Ragama, Sri Lanka and maintained in the insectary of Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya at 26 ˚C and 75± 5 % relative humidity under 12:12 (Light: Dark) photoperiod [34]. Mosquito rearing and colony maintenance were followed according to the guidelines by Standard Operating Procedures (SOP) on rearing and maintenance of *Aedes* mosquito colonies in the insectary by National Research Council, Sri Lanka. The collected eggs were hatched in hatching trays contained 2.0 L of conditioned water. Larvae were fed with diet containing 50 % (w/v) tuna meal (12.5 g), 36 % (w/v) bovine liver powder (9.0 g) and 14 % (w/v) brewer's yeast (3.5 g) dissolved in 100.0 mL distilled water. Approximately 1.5 mL of diet was randomly added into the larval tray. Pupae were collected into bowls after 5 days of eggs hatching using pasture pipettes. Bowls of pupae were placed inside plastic cages (24 x 24 x 24 cm) for adult emergence. Adults were fed with 10 % sucrose solution prepared by dissolving 50.0 g of glucose and 5.0 mL of B - vitamin in 500.0 mL distilled water soaked in cottons. Adult females deprived of sucrose were fed with cattle blood. The five hundred of fourth instar larvae were collected as the test samples from the second generation of the same adults reared under the similar conditions and each concentration was tested for sixty larvae.

Collection of plant materials

Seeds were obtained from five months old matured fruits of *C. spinosa* grown in Ayurveda Herbal Garden, Haldumulla, Sri Lanka (6º45'42" N 80º53'59" E) and the plant was authenticated from National Herbarium, Department of National Botanic Gardens, Peradeniya (ID-43). Collected seeds were air dried for three days and pulverized using an electric grinder to obtain powdered seeds.

Preparation of seed extract

The stock solution of seed extract of *C. spinosa* (SECS) (1000.0 mg L^{-1}) was prepared dissolving 1.0 g of powdered seeds in 1.0 % (v/v) Dimethyl Sulfoxide (DMSO) (≥ 99.5% Sigma-Aldrich, St. Louis, M063178, USA). One percent (v/v) DMSO was prepared mixing 10.0 mL of DMSO and 990.0 mL of water conditioned for one week. The stock solution of SECS was kept overnight for effective extraction of phyto - constituents and filtered through a muslin cloth. The stock solution was diluted to prepare concentrations of 75.0, 125.0, 250.0, 500.0, 1000.0 mg L^{-1} and tested for mortality of *Ae. aegypti* fourth instar larvae.

Larvicidal assay

The assay was conducted according to the guidelines by WHO (2005) [35]. Twenty larvae were released into each container with 200.0 mL of test solutions. Each concentration was replicated at 26 ˚C. Pre-conditioned water was used as the negative control and 1.0 % DMSO was the solvent control which were also triplicated. Both negative and solvent controls were added with twenty larvae in each. Larvae were not fed during the test period to avoid possible interventions that can be caused on the mortality. Number of dead and moribund larvae were counted after 24 h to calculate the mortality.

Statistical analysis

Regression analysis was employed using logit model to compute LC_{50}, Maximum lethal concentration (LC_{90}), 95% fiducial limits of the upper confidence limit (UCL) and lower confidence limit (LCL) using SPSS (Statistical Package of Social Sciences) software version 24.0. A factorial analysis of variance (ANOVA) was performed using concentration as the variable to find the significant difference between that parameter on larval mortality. Larval mortality was served as dependent variable and concentration was treated as the fixed factor.

Analysis of bioactive compounds

Bioactive compounds of SECS were analyzed using preliminary chemical analysis and advanced method of Gas Chromatography Mass Spectrometry (GC-MS).

Preliminary chemical analysis

Phytochemicals contained in twenty grams of powdered seed sample were extracted in to a mixture of 2.0 mL of DMSO and 198.0 mL of pre - conditioned water in a water bath at 45 ˚C for 4 h. The extract was subjected to preliminary phytochemical analysis where the color intensities, color changes and precipitate formations were recorded.

GC-MS analysis

The active chromatographic fraction of the seed extract was further analyzed for phyto - compounds by GC-MS (Model – 7890B, Agilent Technologies, Santa Clara, California, USA). The methodology used by Mdoe *et al*. (2014) was occupied as the reference protocol with modifications for phytochemical analysis [36]. Column (length – 30 m, diameter – 250 µm, thickness - 0.25 µm) was consisted of HP-5MS 5% phenyl Methyl Siloxane and helium was the carrier gas at flow rate of 1.0 mL/min. Detected compounds were identified and relative percentages were clarified using National Institute of Standards and Technology (NIST) library as the external standard database. Retention time and area percentage of each component were evaluated.

Results and discussion

The results exhibited positive correlation between SECS and larvicidal activity with the presence of crucial active compounds.

Larvicidal activity

The SECS exhibited significant larvicidal activity against fourth instar larvae of *Ae. aegypti* within 24 h. The larvae mortality was observed following several structural and behavioral changes. Major structural damages were observed in the head, abdomen and thorax of larvae (Figure 3a).

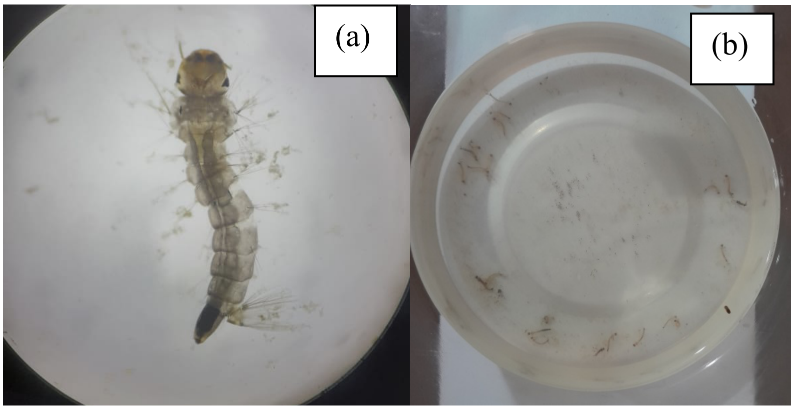

Figure 3: (a) Structurally damaged larvae of *Ae. aegypti* (b) Dead bodies of larvae floated on the surface and settle down at the bottom of the test container

The behavioral changes and the mortality durations depended on the test concentrations. At the beginning of introduction of larvae showed no distinct behavioral changes in any of the concentrations yet nearly after 10 min they restlessly swam between top and the bottom of the solution body, notably in the highest concentration of 1,000.0 mg L^{-1}. After 20 min, restless swimming and the rapid movements were slowed down and the dead bodies started floating on the surface and some settled at the bottom of the container (Figure 3b). Larvae treated in low concentrations showed normal swimming behavior soon after the introduction and attained moribund stage as of increasing the exposure time.

The SECS recorded concentration dependent significant mortality (p = 0.003) with LC_{50} of 233.67 mg L^{-1} (UCL; 282.52 mg L^{-1} – LCL; 184.80 mg L^{-1}) and LC_{90} of 659.93 mg L^{-1} (UCL; 708.85 mg L^{-1} - LCL; 611.07 mg L^{-1}) against fourth instar larvae of *Ae. aegypti* (Figure 4). Mean mortality percentage (MMP) was < 20 % up to the concentration of 125.0 mg L^{-1} and a steep up to 66.66 % starting from 250.0 mg L^{-1} (Table 1). At the highest concentration of 1,000.0 mg L^{-1} all twenty larvae were died within 24 h. Among the scarcely found literatures, the most recent study revealed the larvicidal activity of petroleum ether, ethanol, acetone and water extracts of SECS grown in India against laboratory reared *Ae. aegypti* which reported LC_{50} as 184.257, 465.224, 248.680 and 210.212 mg L^{-1} respectively [33]. They revealed LC_{90} of aqueous seed extract as 3205.89 mg L^{-1}. The recorded LC_{50} of hydroalcoholic seed extract of Sri Lanka (233.67 mg L^{-1}) is slightly higher that of recorded by Anoopkumar *et al*., (2020) and conversely, the LC_{90} of Sri Lankan variety (659.93 mg L^{-1}) is significantly low; more than quarter of the recorded LC_{90} by Indian variety. Based on the results, Sri Lankan variety possesses moderate larvicidal activity which is preferable in developing efficient bio-larvicides as well, as only a lesser concentration is required for the mortality of 90 % of *Ae. aegypti* larvae population. Most of the studies have used solely the chemical solvents in extracting phyto-constituents wherein the present study only used pre-conditioned water along with DMSO <2.0 % (v/v). Roots and stem barks are highly potent sources of secondary metabolites as of most studies have been focused on and reported significant toxic values. Results of our study will provide information to the field where knowledge was lack on SECP.

Table 1: Concentration dependent larvicidal activity of SECS against fourth instar larvae of *Ae. aegypti* after 24

Concentration (mg L^{-1})	MMP ± SD
75.0	6.66 ± 0.88
125.0	18.33 ± 0.88
250.0	66.66 ± 0.88
500.0	81.66 ± 0.88
1000.0	95.00 ± 2.00

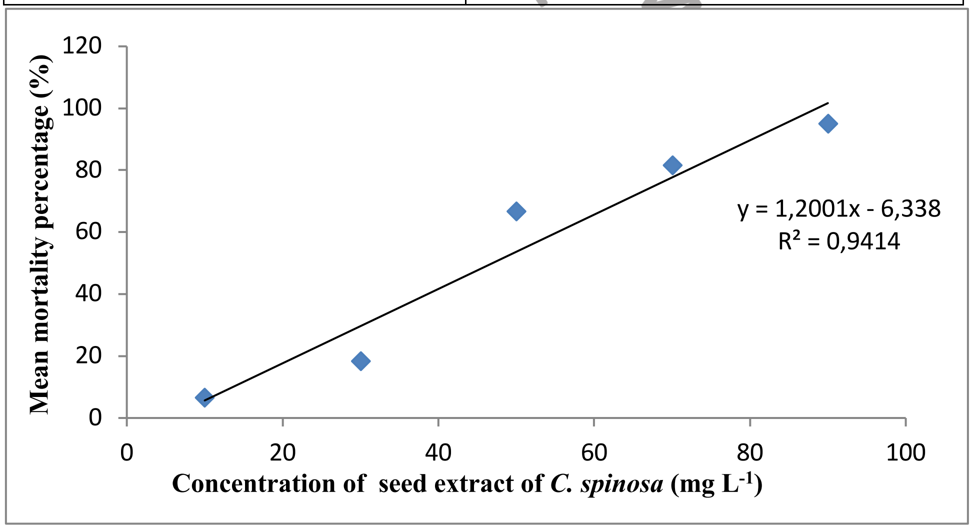

Figure 4: Graphical representation of concentration dependent larvicidal activity of SECS against fourth instar larvae of *Ae. aegypti* after 24 h

Sub - acute toxicity was observed at low concentrations of the extract as the number of larvae developed into pupae was lesser compared to that of in the controls. Sub - acute toxicity can reduce the fertility, fecundity and life span of developed larvae into adults after the few days of their survival. No mortality was observed in the controls instead normal larvae and pupae developments along with the adult emergence after 8 - 9 days of eggs hatching.

Preliminary analysis

The SECS showed positive results for alkaloids, saponins, tannins, flavonoids, coumarins and triterpenes. Insolubility of alkaloids, flavonoids, coumarins, fatty acids and triterpenes in water was overcome by dissolving the powdered seeds in DMSO < 2.0 % (v/v).

GC-MS analysis

Present study identified 19 chemical compounds in SECS using GC-MS analysis after comparing with known compounds in NIST database (Table 2). The chromatogram is presented in Figure 5. Butanoic acid, palmitic acid, n-hexadecanoic acid, octadecanoic acid were the major compounds identified which could be responsible for the larvicidal activity of SECS.

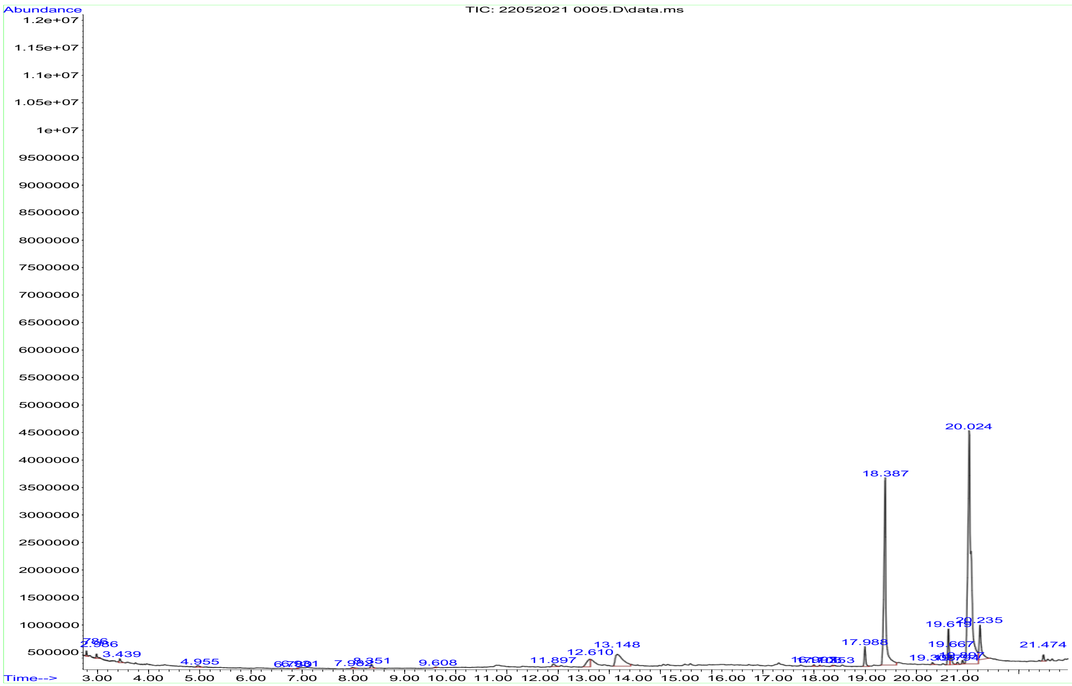

Figure 5: GC-MS chromatogram of phytochemicals detected in SECS using GC-MS analysis

Table 2: Phytochemicals detected in SECS using GC-MS analysis

No.	Retention time (min.)	Compound name
1	2.986	Glycerin
2	3.439	3-methylbutanoic acid/ Isovaleric acid
3	4.955	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	6.731	Unknown
5	6.790	Unknown
6	7.982	Pyranone
7	8.351	Unknown
8	9.608	2-(ethoxymethyl) oxirane
9	11.897	Tetradecanoic acid
10	12.610	2-Methoxy-4-propylphenol/ Ceruglignol

11	13.148	8-methyltetrahydro-4H-(1,3)-dioxino(5,4-d)(1,3) dioxepin-9-ol
12	16.153	2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-
13	17.016	Neophytadiene
14	17.988	Palmitic acid, methyl ester
15	18.387	Hexadecanoic acid, methyl ester
16	19.306	n-Hexadecanoic acid
17	19.897	Phytol
18	20.235	Octadecanoic acid/ oleic acid
19	21.474	Triethylene glycol monododecyl ether

Phytochemical constituents and plausible mechanisms

Generally, classes of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, coumarins, triterpenes, acetogenins, lignans, fatty acids and naphthoquinones stand for larvicidal/ insecticidal activity of the plants [37-41]. Most of these compounds were identified in the SECS grown in Sri Lanka using preliminary and GC-MS analysis. The same compounds were reported for major physiological malformations in the body and to inhibit the nerve signal transmission in the Central Nervous System (CNS) of larvae. Saponin causes stomach toxicity via physiological and morphological damages to midgut epithelial cells of mosquitoes where the excessive absorption causes cells to get vacuolated and vesicles to release energy for detoxification, leading cells to die [42]. Saponin as one of the components might be caused the physical damages observed in the test larvae. Also, saponins lead the retarded development, decreased fecundity and the increased mortality of larvae even after their survival where the similar traits were observed in our test samples. Damaged and disrupted abdomen of larvae and attaining them moribund stage would be caused by the presence of tannins as those are reported in attacking the integrity of digestive track. Because tannins decrease the nutrients intake via hardening the cell membranes and cause malformations due to the excessive ingestion and fumigation of toxicants [43]. Flavonoids as a substance found in the extract might strongly inhibited the Acetyl Cholinesterase (ACh) activity in larvae causing them to paralyze and also to affect their detoxification system by interrupting the activities of Glutathione S - Transferase (GST) and esterase [44]. The detected fatty acids also undergo the same mechanism of inhibiting ACh in CNS [45, 46]. Both tannins and flavonoids are not only neurotoxicants but generate the free radicals to damage the DNA, where the respective formations cause the malformations in proteins resulting mosquitoes to die [33]. As of SECS is reported with significant level of free radicals that can be caused DNA damages of larvae [33]. The synergetic effect of neurotoxicity and the free radical generation can be one of the reasons for death of *Ae. aegypti* larvae. Also, the identified compound composition with several pharmacological activities including anti-microbial activity might enhanced the productivity of the extract as it diminishes the host-parasite interaction in the mosquito midgut. However, a combined chemical composition with multiple properties is more effective than using a single or few compounds with limited activities for mosquito management strategies which is well-employed in the SECS extract.

In the current local market, there is already a bacterium-based product (*Saccharopolyspora spinose*) but not an ethnobotanically developed formulations where the produced knowledge in the present article will raise the competition of the natural ingredients-based products to discourage the use of synthetic mosquitocides, and finally to make mosquito control in Sri Lanka hassle-free and effective. Also, the unveiled information will allow to get the maximum value of a well-grown yet underrated medicinal plant in Sri Lanka.

Impact on non-target organisms

Field trials are to be performed on the non-target organisms and none of the literatures mentioned the side effects of SECS over unbalancing of the ecosystem or undesirable effects on non-target organisms and human health. Supporting the use of less toxic SECS, a highly toxic chloroform seed extract of *Annona mucosa* which contains *squamosin* as the larvicidal compound (LC_{50}: 0.01 mg L^{-1}) showed no toxic effect on two predators of *Ae. aegypti* - *C. bigoti* and *Toxorhynchites theobaldi* and also to the mammals based on the results against human leukocytes [47].

Conclusion

Mortality of *Ae. aegypti* larvae exposed to different concentrations of SECS was concentration dependent and significant which recorded LC_{50} of 233.67 mg L^{-1} and LC_{90} of 659.93 mg L^{-1} possibly due to the presence of saponins, alkaloids, coumarins, tannins, flavonoids and fatty acids. All these results explicit the naturally occurring larvicidal potential of an underrated medicinal plant of *C. spinosa* to be successfully use as a safe alternative for chemical larvicides to combat the dengue mosquitoes and for a sustainable health system of Sri Lanka amidst of promoting nature-based products.

Future direction

Indiscriminate use of chemical adulticides and larvicides has become a massive disaster in terms of both environmental and human sustainability. Unfortunately, chemical mosquitocides are favored by users due to user-friendly approach, effectiveness and long-lasting activity which is affirmed by different product modifications. Unlike chemical products, if a bio-larvicide is developed using SECS will be easily degradable, less harmful to both users and non-target species and will be low probability in developing resistance from target species. Even though, the larvicides derived from natural resources are safer than the chemical products, it is concerning as the conventional use of whole plant would affect the diversity and the conservation rationale of the plant as mass usage of raw materials. Anyhow, unique and optimized protocols for extracting target compounds from *C. spinosa* will allow to use only small quantities. Also, usage of suitable adjuvants as buffering and antimicrobial agents, UV protectant, emulsifiers, stabilizers and for adhesion will ensure the firmness and durability of *C. spinosa* based product facilitating to develop cost-effective and eco-friendly bio-larvicides. As the reported LC_{90} of SECS was noticeably low, a competitive and commercially worth bio-larvicide can be developed. Further investigations on optimum concentration and applying frequencies will improve the product efficacy and the survival in different environmental conditions. Further, the future direction of using *C. spinosa* can be oriented for field trials in studying persistency of the formulation and its effectiveness, effects of non-target species such as important worms, snails and small insects etc. Studying the effects on soil conditions such as soil pH and sterility will be important in mitigate the side effects of the product.

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Authors' contribution

PK Lawrence: Conceptualization, Writing - Original Draft, Review & Editing. WTPSK Senarath: Writing - Review & Editing, Supervision. M.D. Hapugoda: Writing - Review & Editing, Supervision.

Statements and declarations

Ethical considerations

Not applicable.

Declaration of conflicting interest
Authors have declared that no competing interests exist.
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Data availability
No data was used for the research described in the article.
Disclaimer
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