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Review Article

Biochemical and Entomological Validation of Spathoda Campanulata Q As A Bioenhancer in Plant-Based Mosquito Control Strategies

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A B S T R A C T

Mosquito-borne diseases such as dengue and malaria remain pressing public health concerns, driven by rising resistance to conventional insecticides. This study evaluates the larvicidal and repellent efficacy of *Spathoda campanulata* Q (homeopathic mother tincture), prepared by macerating fresh flowers in 90% ethanol. Phytochemical profiling through TLC, UV-Vis (λmax at 275 nm and 325 nm), FTIR, and GC–MS revealed key bioactives including phenol (1.67%), 1,2-benzenediol (3.56%), benzofuran (5.13%), hexadecanoic acid ethyl ester, and n-tetracosanol-1 (6.32%). These compounds are known to exert neurotoxic, antioxidant, and cuticle-disruptive actions on mosquito larvae. FTIR confirmed the presence of larvicidally relevant functional groups: hydroxyl, amine, carbonyl, aromatic C=C, and ether stretches. Larvicidal bioassays against third instar *Aedes aegypti* and *Anopheles stephensi* demonstrated LC_{50} values of 75.20 ppm and 74.40 ppm, respectively, for *Spathoda* Q, with LC_{90} values of 122.0 ppm and 123.0 ppm—comparable to 1% Azadirachtin ($p > 0.05$). Enzyme inhibition assays showed dose-dependent suppression of acetylcholinesterase (IC_{50}: 489.16 ppm) and glutathione S-transferase (IC_{50}: 670.12 ppm), implicating neuroinhibitory and detoxification pathways in its mode of action. A cage test comparing two vaporizer formulations (with and without *Spathoda* Q) demonstrated significantly greater repellency ($p < 0.001$) in the *Spathoda*-based formulation, with knockdown at 91.58%, 24-hour mortality at 88.41%, and repellency up to 96.12% at 2 hours. The formulation remained stable over 30 days. These findings confirm that *Spathoda campanulata* Q is a potent, eco-friendly botanical suitable for incorporation into integrated mosquito management strategies.

Keywords: Acetylcholinesterase, Bioinsecticide, Detoxification enzymes, Phytochemical profiling, Vaporizer formulation

Introduction

Vector-borne diseases such as malaria, dengue, chikungunya, and filariasis continue to exert a significant burden on global public health, particularly across tropical and subtropical countries like India.1 Mosquitoes, especially *Aedes aegypti* and *Anopheles stephensi*, are among the primary vectors responsible for the transmission of these diseases.2 The World Health Organization (WHO) estimates that over 3.9 billion people in more than 120 countries are at risk of dengue alone, with millions of malaria cases being reported annually.3 These public health challenges are exacerbated by rapid urbanization, climate change, and increasing resistance of mosquito vectors to conventional chemical insecticides.4 Traditional mosquito control strategies have predominantly relied on synthetic larvicides and repellents including organophosphates, pyrethroids, and carbamates.5 While these chemicals offer rapid knockdown and high mortality rates, their continued usage has led to critical issues such as environmental pollution, bioaccumulation in non-target organisms, carcinogenic potential, and development of resistance among mosquito populations.6 Furthermore, synthetic repellents like DEET (N,N-Diethyl-meta-toluamide) have raised safety concerns related to dermal irritation, neurotoxicity, and long-term exposure risks.7 These limitations underline the urgent need for safer, sustainable, and eco-friendly alternatives to existing vector control tools.

In recent years, considerable attention has turned toward botanical-based insecticides and repellents, which offer several advantages such as biodegradability, target specificity, and lower ecological toxicity.8 Phytochemicals derived from medicinal plants have demonstrated broad-spectrum bioactivity against mosquito larvae and adults.9 They interfere with multiple biological pathways, including neuromuscular coordination, hormonal regulation, and detoxification mechanisms, making them less susceptible to resistance development.10 Despite the growing body of literature on plant-based larvicides and repellents, relatively few studies have explored the synergistic or additive effects of combining multiple phytoconstituents into a single standardized formulation.11 One such underutilized plant with promising insecticidal potential is *Spathoda campanulata* (syn. *Spathodea campanulata*), commonly known as the African tulip tree. It is a member of the Bignoniaceae family, native to tropical Africa but widely cultivated across Asia, particularly in India, for ornamental and shade purposes.12 Traditionally, the bark, flowers, and leaves of *Spathoda campanulata* have been used in indigenous systems of medicine for treating wounds, fevers, infections, and inflammatory conditions.13 The phytopharmacological profile of this plant includes a diverse range of secondary metabolites such as flavonoids, alkaloids, phenolics, tannins, and fatty acid derivatives many of which possess documented insecticidal, antimicrobial, and antioxidant properties.

The potential application of *Spathoda campanulata* in vector control remains largely untapped, particularly in the form of standardized extracts or formulations. While preliminary ethnobotanical and phytochemical reports suggest its suitability for mosquito management, there is a lack of robust scientific studies that systematically evaluate its efficacy and mechanisms of action. Specifically, the homeopathic mother tincture (Q potency) of *Spathoda campanulata*, prepared using hydroethanolic maceration, retains a complex profile of bioactive constituents and is widely recognized in integrative medicine for its safety, stability, and affordability. Its application in the domain of vector control, however, remains unexplored. Given the rising demand for plant-based vaporizer formulations that combine repellency and larvicidal effects, integrating Spathoda campanulata Q into a herbal repellent platform holds considerable promise. Botanical formulations with multiple modes of action are known to exhibit greater efficacy and durability under field conditions.14 The concept of using *Spathoda campanulata* Q as a bioenhancer or synergistic component in such formulations is particularly novel and may offer a practical solution for community-level vector control interventions. Moreover, the combination of *Spathoda campanulata* with established agents like azadirachtin (from *Azadirachta indica*) a known bioinsecticide may enhance efficacy while reducing dependence on any single active compound.

In the context of mosquito biology, two enzymatic pathways are especially relevant for evaluating larvicidal effects: acetylcholinesterase (AChE), which regulates synaptic transmission in the nervous system, and glutathione-S-transferase (GST), which is involved in detoxification processes.15 Botanicals that inhibit these enzymes have demonstrated efficacy in reducing larval survival and preventing resistance. The presence of specific functional groups such as hydroxyls, carbonyls, and aromatics in plant extracts has been associated with such enzyme inhibition.16 Therefore, analyzing the functional group profile through Fourier Transform Infrared (FTIR) spectroscopy, and the chemical fingerprint via Gas Chromatography Mass Spectrometry (GC–MS), is essential to understand the mechanistic underpinnings of bioactivity. Furthermore, vaporizer formulations intended for mosquito repellency must demonstrate sustained action across time intervals and species, necessitating structured bioassays such as cage tests.17 Key endpoints in such evaluations include knockdown time at 30 and 60 minutes, mortality at 24 hours, and repellency at 2 and 6 hours post-application. Comparative testing of formulations with and without the

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candidate botanical allows for direct assessment of its functional contribution to the overall efficacy.

The need for this study is therefore two-fold. Firstly, it addresses the scientific gap in validating *Spathoda campanulata* Q as a bioactive agent against mosquito vectors. Secondly, it responds to a practical demand for integrated, eco-friendly vector control tools that can complement or replace conventional chemical insecticides.18 By adopting a multidisciplinary approach encompassing phytochemistry, vector biology, toxicology, and formulation science this study seeks to establish a scientific foundation for incorporating *Spathoda campanulata* Q into next generation herbal repellents and larvicides.19 In an era marked by climate-sensitive vector proliferation and growing consumer awareness regarding chemical exposure, there is a compelling case for advancing research on botanical alternatives that align with the principles of sustainability, safety, and efficacy.[20] *Spathoda campanulata* represents an underutilized yet potent botanical with the potential to bridge this gap. Scientific validation of its properties could pave the way for its inclusion in public health programs focused on integrated vector management, especially in endemic settings where traditional and modern approaches must coalesce for maximum impact

Materials and Methods

Materials

The materials used in this study included *Spathoda campanulata* Q mother tincture, prepared by macerating fresh flower in 90% ethanol as per the Homoeopathic Pharmacopoeia of India standards. Azadirachtin (1% w/v), serving as the standard botanical larvicide, was procured from a certified agricultural supplier. Other components used in the herbal vaporizer formulation were isopropyl alcohol (5% w/v) as solubilizer, camphor (2% w/v) and eucalyptus oil (2% w/v) as synergistic repellents, polyethylene glycol 400 (3% w/v) as a co-solvent, and distilled water as the base solvent to make up the volume. TLC reagents included methanol, ethyl acetate, and distilled water (6:3:1), and silica gel F254 plates (Merck, India). FTIR and GC–MS spectral analyses were conducted using PerkinElmer and Agilent 7890B systems, respectively. The larvicidal and repellent assays used third instar larvae and adult mosquitoes of *Aedes aegypti* and *Anopheles stephensi*, obtained and maintained at Alpha Omega Research Foundation, Salem, under standard insectary conditions (27 ± 2°C, 70 ± 5% RH, 12:12 light-dark cycle). Assay kits for acetylcholinesterase (AChE) and glutathione-S-transferase (GST) enzyme activity were purchased from Sigma-Aldrich (USA). All glassware, mosquito cages, micropipettes, solvents, and safety assay kits conformed to laboratory-grade or analytical-grade standards.

Methodology

Preparation of *Spathoda campanulata* Q Mother Tincture

Fresh flowers of *Spathoda campanulata* were collected from a verified botanical source and authenticated a certified taxonomist, and the plant was verified as *Spathodea campanulata* P. Beauv. with the authentication code S290224121C. The mother tincture was prepared using the maceration method described in the Homoeopathic Pharmacopoeia of India (HPI). The flowers were coarsely powdered and macerated in 90% ethanol at a drug to solvent ratio of 1:10 (w/v) for 21 days with intermittent shaking. The extract was filtered, stored in amber glass bottles, and labeled as *Spathoda campanulata* Q.

Physicochemical Characterization

The *Spathoda campanulata* Q mother tincture underwent physicochemical characterization to ensure its quality and suitability for formulation development. The pH was measured using a calibrated digital pH meter at room temperature (25 ± 2°C) after standard buffer calibration. Specific gravity was determined using a 50 ml pycnometer following pharmacopeial procedures, providing insight into the density and concentration of the tincture. Organoleptic evaluation was also performed, including visual assessment of color, odor, and consistency. The tincture was clear, exhibited a characteristic herbal-alcoholic scent, and showed no signs of phase separation or turbidity. These assessments ensured reproducibility and batch-to-batch uniformity for subsequent bioactivity and formulation studies.[21]

Phytochemical Analysis – TLC, UV, FTIR, and GC-MS

The *Spathoda campanulata* Q mother tincture was analyzed using a series of phytochemical techniques to identify its functional groups, active constituents, and overall chemical profile. Thin Layer Chromatography (TLC) was performed using silica gel F254 plates and a solvent system composed of ethyl acetate:methanol:water in a 6:3:1 ratio. The developed chromatograms were visualized under ultraviolet light at 254 nm and 366 nm, as well as exposed to iodine vapors to detect a range of phytochemical constituents based on Rf values and color changes. UV–Visible spectrophotometry was conducted with a Shimadzu UV-1800 spectrophotometer, scanning from 200 nm to 800 nm to detect specific absorption peaks indicative of flavonoids, phenols, and other conjugated compounds. Fourier Transform Infrared (FTIR) spectroscopy was used

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to identify characteristic functional groups. Using the KBr pellet method, the tincture sample was scanned in the range of 4000–400 cm^{-1}, revealing peaks corresponding to hydroxyl, carbonyl, alkene, and aromatic structures. Gas Chromatography–Mass Spectrometry (GC–MS) analysis was carried out on an Agilent 7890B GC system equipped with a DB-5MS capillary column and electron ionization mode. The oven temperature was programmed from 50°C to 280°C at a ramp rate of 10°C/min, and the injection port was maintained at 250°C.[21] Detected peaks were interpreted using the NIST database to identify major phytochemical compounds present in the tincture. Together, these techniques provided a comprehensive profile of the bioactive molecules in *Spathoda campanulata* Q, supporting its potential role as a larvicidal and mosquito-repellent agent.

Larvicidal Bioassay

The larvicidal potential of *Spathoda campanulata* Q was evaluated using early fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*. A series of test concentrations of the mother tincture were prepared and added to 250 ml glass beakers containing 100 ml of water. Twenty-five larvae were introduced into each beaker, and the setup was maintained at 28 ± 2°C with continuous monitoring. Each concentration was tested in quadruplicate, and the larvae were provided with standard larval food. A negative control (solvent only) and a positive control containing 1% Azadirachtin were included. Mortality was recorded after 24 hours of exposure, and the results were expressed as percentage mortality. Non-motile and moribund larvae were considered dead. Subsequently, live pupae were transferred to fresh water, and cumulative larval and pupal mortality was tracked daily. LC_{50} and LC_{90} values were determined using probit analysis.[22]

Biochemical Enzyme Assays (AChE and GST Activity)

To investigate the neurotoxic and detoxification-related biochemical effects of *Spathoda campanulata* Q, larval acetylcholinesterase (AChE) and glutathione S-transferase (GST) enzyme activities were assessed. For the AChE assay, five larval heads from *Aedes aegypti* and *Anopheles stephensi* were dissected and homogenized in a buffer solution containing 1 ml of Triton X-100, 38.03 mg ethylene glycol tetraacetic acid (EGTA), 5.845 g NaCl, and 80 ml Tris buffer (10 mM, pH 7.0). The homogenate was centrifuged at 5000 rpm for 5 minutes at 4°C, and 100 µl of the resulting supernatant was added to a reaction mixture containing 1 ml of 0.1 M Tris buffer (pH 8.0) and 100 µl of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). After an initial reaction period of 5 minutes, 100 µl of acetylthiocholine iodide was added as the substrate. The enzymatic hydrolysis of acetylthiocholine was monitored at 412 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). A reduction in absorbance compared to controls indicated AChE inhibition by the test sample.For GST activity, decapitated larvae were homogenized in 0.1 M sodium phosphate buffer (pH 6.0), followed by centrifugation at 10,000 rpm for 30 minutes at 4°C. The supernatant was used to assess GST activity by incubating with CDNB (1-chloro-2,4-dinitrobenzene) and reduced glutathione (GSH). The conjugation reaction was monitored by measuring absorbance at 340 nm. The protein concentration of the homogenates was determined using the Bradford assay (1976), with bovine serum albumin (BSA) as the standard. Enzyme activity was expressed in terms of nmol/min/mg protein, and percentage inhibition was calculated relative to the control group.[23]

Formulation of Herbal Vaporizer and its Preparation Method

The herbal vaporizer formulations were prepared to assess the enhanced efficacy of *Spathoda campanulata* Q in combination with other established repellent agents. Two formulations were developed: one containing *Spathoda campanulata* Q and another without it to serve as a comparative control. For the test formulation, *Spathoda campanulata* Q was incorporated at 15% w/v as the primary homeopathic ingredient. Azadirachtin (Neem extract) at 1% w/v was included as the primary active repellent agent. To enhance solubility and provide antiseptic properties, 5% w/v isopropyl alcohol was used. Camphor and eucalyptus oil were included at 2% w/v each to serve as synergists and additional volatile repellents. Polyethylene glycol 400 (3% w/v) acted as a co-solvent and stabilizer. The final volume was adjusted using distilled water to achieve a 100 ml formulation. The preparation involved sequential mixing under aseptic conditions.[24] All liquid ingredients were first measured and mixed under magnetic stirring at ambient temperature to ensure homogeneity. The final mixture was stored in amber-colored vaporizer-compatible bottles at room temperature away from direct sunlight. The control formulation followed the same composition excluding *Spathoda campanulata* Q.

Stability Test

To evaluate the physicochemical stability and shelf-life suitability of the formulated herbal vaporizer, stability studies were conducted over a period of 30 days. Both formulations with and without *Spathoda campanulata* Q were stored at three different conditions: room temperature (25 ± 2°C), refrigerated temperature (4 ± 1°C), and accelerated condition (40 ± 2°C with 75% RH). Samples were periodically examined on Day 0, Day 15, and Day 30 for changes in physical appearance (color, clarity, and phase separation), pH, and odor. Any signs of precipitation,

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discoloration, or volatility loss were recorded. The pH of each sample was measured using a digital pH meter, and the formulations were also visually checked for turbidity or phase instability.[24] No additional stabilizers were added during the test period. The results of these observations determined the formulation's compatibility and retention of functional attributes under different environmental conditions, supporting its practical application as a room vaporizer.

Cage Repellency Test

The repellency efficacy of the formulated herbal vaporizer was assessed using a cage-based mosquito repellency test under controlled laboratory conditions. Adult female *Aedes aegypti* and *Anopheles stephensi* mosquitoes (5–7 days old and non-blood fed) were used for the experiment. The test was conducted in a ventilated mosquito cage measuring 60 × 40 × 40 cm. For each test, 20 female mosquitoes were introduced into the cage, and a cotton pad soaked with 10 ml of the vaporizer formulation (with or without *Spathoda campanulata* Q) was placed inside. The vaporizer pad was allowed to diffuse its contents for 10 minutes before introducing the mosquitoes. Repellency was evaluated by observing mosquito landing and resting behavior every 10 minutes over a 60-minute period. The number of mosquitoes repelled (i.e., those avoiding the treated area or showing agitated flight) was recorded and compared to both the control formulation and a positive control using commercially available neem-based vaporizer.[24] Percent repellency was calculated using the standard formula:

$$Percent\ Repellency = \frac{C - T}{C} X\ 100$$

where *C* is the number of mosquitoes in control and *T* is the number in treated group.

Statistical Analysis

All experimental procedures were performed in triplicate unless otherwise specified. Data from larvicidal bioassays, enzyme inhibition studies (AChE and GST), physicochemical stability tests, and cage repellency experiments were expressed as mean ± standard deviation (SD). Probit analysis was conducted using SPSS version 26.0 to determine the LC_{50} and LC_{90} values for larvicidal efficacy against *Aedes aegypti* and *Anopheles stephensi*. Differences between the formulations (with and without *Spathoda campanulata* Q) were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant.

Results:

The *Spathoda campanulata* Q mother tincture exhibited a pH of 6.50 ± 0.40 and a specific gravity of 1.04 ± 0.01 g/cm³, indicating a slightly acidic and moderately dense hydroethanolic preparation, suitable for vaporizer and topical applications. Organoleptically, the formulation was transparent and light brown in appearance, with a characteristic aromatic odor and good solubility. Phytochemical investigations through TLC revealed three distinct bands under UV light, suggesting the presence of moderately polar secondary metabolites such as flavonoids, alkaloids, or glycosides. UV-Visible spectral analysis showed two primary absorbance peaks at 275 nm and 325 nm, typically attributed to aromatic phenolic compounds and flavonoid moieties. (Figure.1) FTIR spectral analysis further confirmed functional groups with biological relevance, such as hydroxyl (O–H), amine (N–H), carbonyl (C=O), aromatic C=C, and C–O stretches, which are often implicated in antioxidant, neurotoxic, and enzymatic inhibition mechanisms. (Figure.2) GC–MS profiling identified bioactive compounds including phenol, 1,2-benzenediol, benzofuran derivatives, ethyl esters of long-chain fatty acids, and high-molecular-weight hydrocarbons such as cyclotetracosane and n-tetracosanol, all of which have documented larvicidal, neuroinhibitory, or repellent effects. (Figure.3)

In the larvicidal bioassay, *Spathoda campanulata* Q induced concentration-dependent mortality in fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*. Probit analysis estimated the LC_{50} values as 75.20 ppm and 74.40 ppm and LC_{90} values as 122.00 ppm and 123.00 ppm for *Aedes* and *Anopheles* respectively, indicating potent larvicidal action. Although Azadirachtin (1%), used as the positive control, exhibited marginally lower LC values, statistical comparison showed no significant difference ($p > 0.05$), thus affirming comparable efficacy of *Spathoda* Q. (Figure.4) Biochemical assays targeting acetylcholinesterase (AChE) and glutathione S-transferase (GST) demonstrated dose-dependent enzyme inhibition by both samples. ANOVA revealed statistically significant inhibition based on concentration for both enzymes ($p < 0.001$), with minimal effect due to sample type. IC_{50} analysis showed that *Spathoda* Q inhibited AChE at 489.16 ppm, while Azadirachtin required a higher concentration (1030.74 ppm) for comparable inhibition, suggesting a steeper and more effective inhibition curve for *Spathoda*. In GST assays, both samples showed near-equivalent IC_{50} values (670.12 ppm for *Spathoda* and 677.57 ppm for Azadirachtin), confirming potent detoxification pathway interference. (Figure.5)

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In cage repellency tests comparing two formulations—Formulation A (with *Spathoda* Q) and Formulation B (without *Spathoda*)—the inclusion of *Spathoda* Q resulted in significantly improved knockdown, mortality, and repellency rates across both mosquito species. At 30 and 60 minutes, knockdown rates for Formulation A reached 76.17% and 91.58% in *Aedes* and 70.32% and 88.63% in *Anopheles* respectively, while 24-hour mortality reached 88.41% and 83.41%. Repellency at 2 hours post-exposure was 96.12% for *Aedes* and 93.35% for *Anopheles*, and was sustained at 6 hours with 84.74% and 78.22% respectively. These differences were statistically significant ($p < 0.001$) when compared to Formulation B, confirming that *Spathoda* Q notably augments repellent activity in the vaporizer. (Figure.6) Stability evaluation over 30 days revealed no changes in pH, color, or physical integrity in either formulation under varied storage conditions, with pH values ranging from 6.4 to 6.6. This stability data further supports the feasibility of incorporating *Spathoda campanulata* Q into herbal vaporizers for long-term use. Collectively, these findings validate the larvicidal and repellent potential of *Spathoda* Q, offering a plant-based, eco-compatible solution for mosquito control.

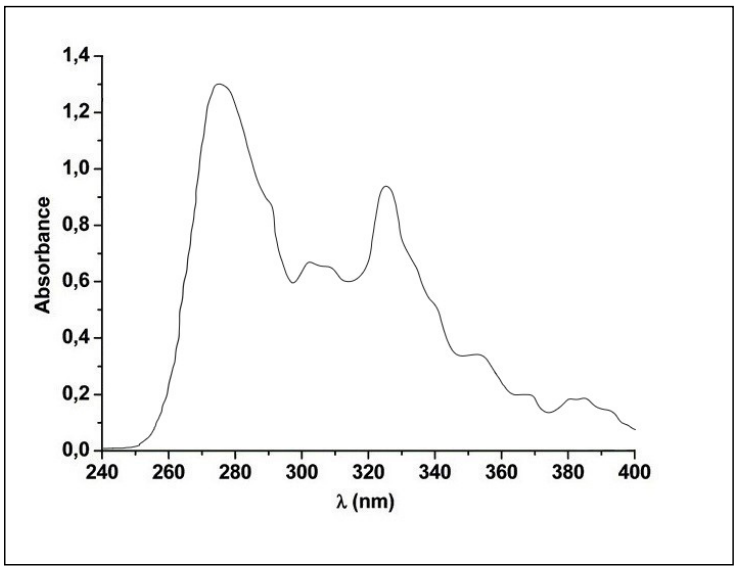

Figure 1.UV–Visible spectrum of *Spathoda campanulata* Q showing peaks at ~275 nm and ~325 nm, indicating the presence of phenolic and flavonoid compounds linked to its antioxidant and bioactive potential

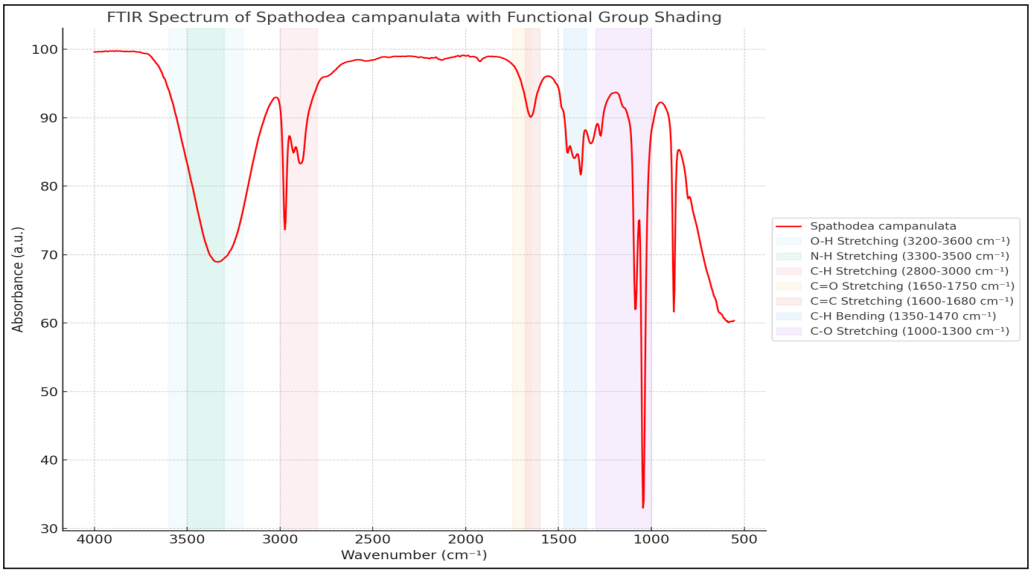

Figure 2.FTIR spectrum of *Spathodea campanulata* Q highlighting major functional groups including O–H (3200–3600 cm^{-1}), N–H (3300–3500 cm^{-1}), C–H (2800–3000 cm^{-1}), C=O (1650–1750 cm^{-1}), C=C (1600–1680 cm^{-1}), C–H bending (1350–1470 cm^{-1}), and C–O (1000–1300 cm^{-1}), which contribute to its larvicidal and repellent bioactivity

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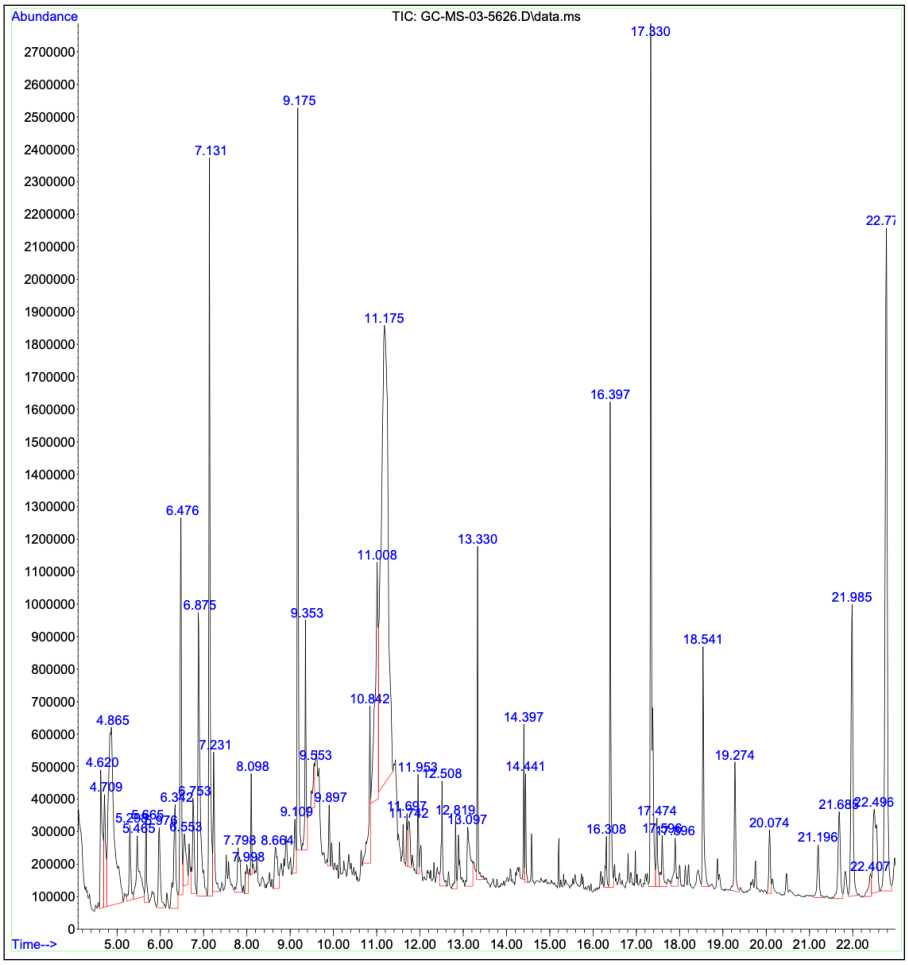

Figure 3.GC–MS total ion chromatogram (TIC) of *Spathodea campanulata* Q extract showing major peaks at retention times 4.62, 6.476, 7.131, 9.175, 11.175, 13.330, 17.330, and 18.541 minutes. These peaks correspond to bioactive compounds such as phenol, 1,2-benzenediol, benzofuran, and fatty acid esters with known insecticidal, larvicidal, and repellent activities

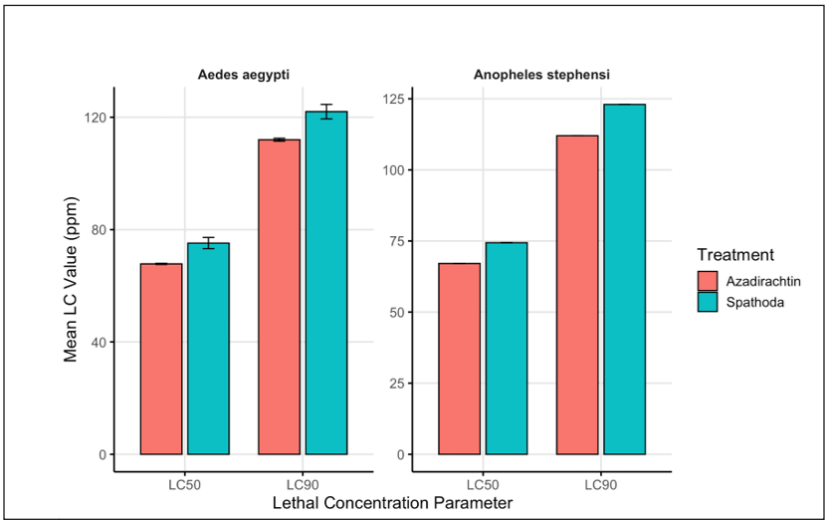

Figure 4.Mean LC_{50} and LC_{90} values of *Spathoda* Q and Azadirachtin 1% against *Aedes aegypti* and *Anopheles stephensi*. Both agents showed comparable larvicidal activity with minor, non-significant differences

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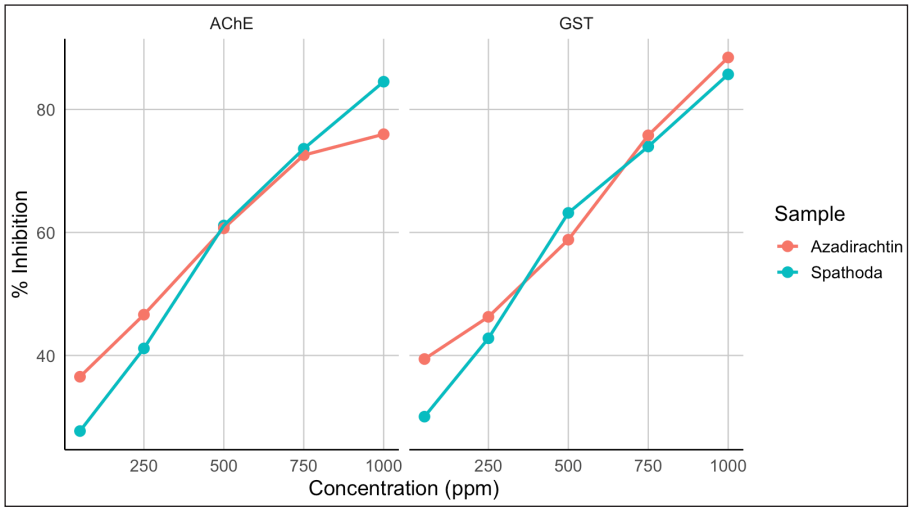

Figure 5.Dose-dependent enzyme inhibition (%) of AChE and GST by *Spathoda campanulata* Q and 1% Azadirachtin across concentrations (100–1000 ppm). Spathoda shows a higher AChE inhibition at 1000 ppm, while both samples exhibit comparable GST inhibition, indicating Spathoda's potent enzymatic suppression at elevated doses

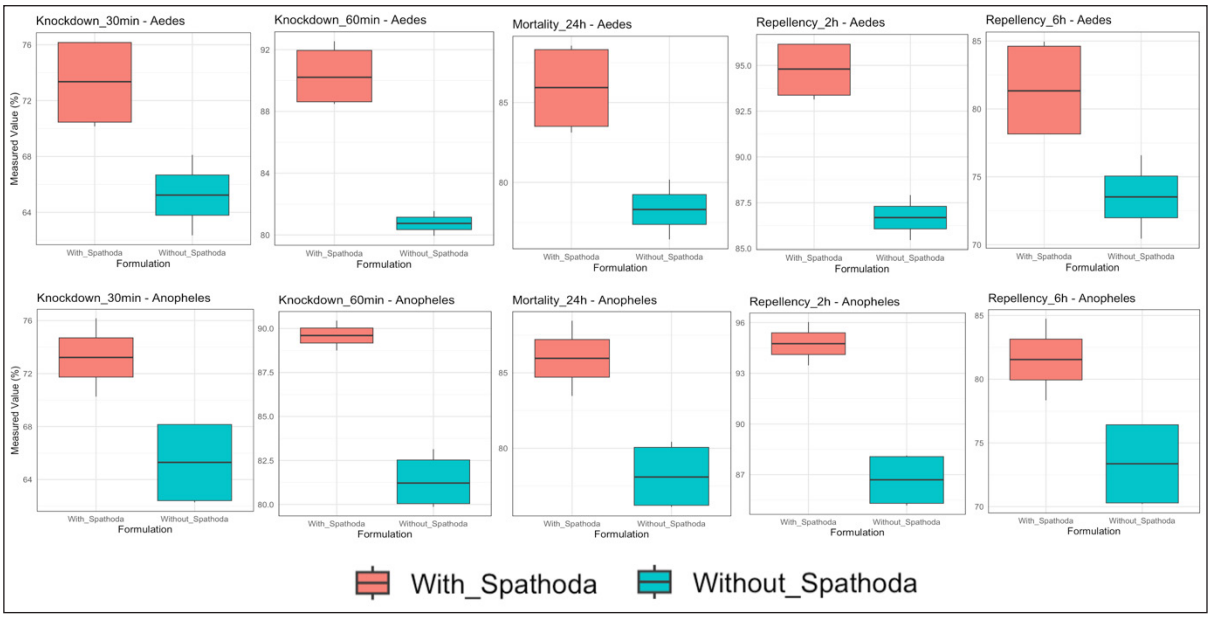

Figure 6.Boxplot comparison of knockdown, mortality, and repellency metrics between herbal vaporizer formulations with and without *Spathoda campanulata* Q. The inclusion of Spathoda significantly enhanced all entomological outcomes for both *Aedes aegypti* and *Anopheles stephensi*.

Discussion

The present investigation explored the insecticidal and repellent efficacy of *Spathoda campanulata* Q, a homeopathic mother tincture, with a primary focus on its phytochemical profile and biochemical interactions relevant to mosquito control. Of particular significance is the array of bioactive compounds identified through GC–MS analysis, which serve as a mechanistic basis for the observed larvicidal and enzyme-inhibitory activities. Among the notable compounds, phenol derivatives such as 1,2-benzenediol (catechol), benzofuran, and various fatty acid ethyl esters emerged prominently. These constituents are increasingly recognized in vector control studies due to their dual roles in neurotoxic interference and cuticle-disrupting action in mosquito larvae.[25] Catechol-based phenolics are known for their oxidative and cytotoxic properties, potentially targeting larval nervous systems through redox cycling and reactive oxygen species generation.[26]which address the mechanism of antioxidant activity and focus on the kinetics of the reactions including the antioxidants. Many studies have been conducted with evaluating antioxidant activity of various samples of research interest using by different

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methods in food and human health. These methods were classified methods described and discussed in this review. Methods based on inhibited autoxidation are the most suited for termination-enhancing antioxidants and, for chain-breaking antioxidants while different specific studies are needed for preventive antioxidants. For this purpose, the most commonly methods used in vitro determination of antioxidant capacity of food and pharmaceutical constituents are examined and also a selection of chemical testing methods is critically reviewed and highlighting. In addition, their advantages, disadvantages, limitations and usefulness were discussed and investigated for pure molecules and raw plant extracts. The effect and influence of the reaction medium on performance of antioxidants is also addressed. Hence, this overview provides a basis and rationale for developing standardized antioxidant capacity methods for the food, nutraceuticals, and dietary supplement industries. Also, the most important advantages and shortcomings of each method were detected and highlighted. The underlying chemical principles of these methods have been explained and thoroughly analyzed. The chemical principles of methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH• Benzofuran, a heterocyclic compound found in numerous plant-based larvicides, has been demonstrated to disrupt neurotransmitter function and synaptic transmission, suggesting its role in interfering with acetylcholinesterase activity a hypothesis further supported by our biochemical assay data.[27] The presence of long-chain fatty acid ethyl esters and aliphatic hydrocarbons such as n-tetracosanol-1 and cyclotetracosane in the GC–MS profile introduces additional dimensions to the larvicidal and repellent potential of *Spathoda campanulata*. Fatty acid esters are biologically significant not only for their membrane-disruptive effects but also for their ability to interfere with hormonal regulation during insect development.[28] These lipophilic compounds can impair larval molting and cuticle formation, ultimately leading to growth arrest or incomplete pupation. Furthermore, their volatility and surface activity enhance the formulation's efficacy when used in a vaporizer matrix, enabling a sustained release of repellent agents into the surrounding airspace.[29]solvents, and nicotine. Heating this liquid generates an aerosol that is inhaled into the lungs in a process commonly referred to as vaping. E-cigarette devices can also contain cannabis-based products including tetrahydrocannabinol (THC Long-chain alcohols and hydrocarbons such as tetracosanol and cyclotetracosane are often associated with feeding deterrence and olfactory disruption in insects.[30] Their presence may contribute to a multi-pronged repellent effect by masking host cues or interfering with the mosquito's chemosensory system.

What sets this study apart is the concurrent evaluation of enzyme inhibition activity in mosquito larvae, which provides direct evidence of physiological interference. The use of acetylcholinesterase (AChE) and glutathione S-transferase (GST) assays serves as a proxy for neurotoxicity and detoxification pathway engagement, respectively. AChE, a key enzyme in the neural signaling pathway, is a classical target for organophosphates and carbamate insecticides, but its inhibition by botanical extracts underscores the emerging relevance of phytochemical alternatives.[31]organophosphates have common applications in pesticides and herbicides, as well as nerve agents in chemical warfare. Therefore, most patients exposed to organophosphates typically encounter these compounds through the use of insecticides and herbicides. When introduced into the body, organophosphates inhibit the enzyme acetylcholinesterase (AChE In this study, *Spathoda campanulata* Q demonstrated substantial inhibition of AChE activity, indicative of its potential to elicit rapid knockdown effects by disrupting cholinergic transmission. This aligns with the suspected role of phenolic and heterocyclic components in blocking or degrading the synaptic enzyme, leading to neuromuscular paralysis and eventual mortality.[32]optimize surgical conditions, and assist with mechanical ventilation in patients with reduced lung compliance. Neuromuscular blocking agents (NMBAs The inhibition of GST, another critical enzyme involved in detoxification of xenobiotics within insect tissues, suggests a secondary mechanism of toxicity.[33]glutathione S-transferases (GSTs GST enzymes protect insect larvae by neutralizing oxidative stress and metabolizing insecticides; their inhibition compromises the larval ability to defend against phytochemical exposure.[34] The moderate-to-potent GST inhibition observed in this study indicates that *Spathoda campanulata* Q not only exerts a neurotoxic effect but also impairs the larvae's metabolic resilience, potentially enhancing the efficacy of both its own constituents and other co-applied agents like Azadirachtin. This dual inhibition pathway is significant because it underscores the broad-spectrum vulnerability induced in the larval population, reducing the likelihood of resistance development through singular metabolic adaptation.

It is also important to reflect on the broader ecological implications of these findings. The emergence of insecticide resistance and the environmental burden of synthetic larvicides have accelerated the search for natural alternatives.[35] The composition of *Spathoda campanulata* Q presents a compelling case for its role in integrated vector management strategies. Unlike single-compound insecticides, botanical preparations typically contain a synergistic blend of phytochemicals that act on multiple physiological targets. This complexity reduces the selective pressure for resistance and offers a more sustainable approach to vector control.[36]Bemisia tabaci, is an invasive polyphagous pest affecting several agricultural

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and horticultural crops worldwide. A multimodal effort involving mixing new-generation chemicals and botanical insecticides effectively eliminate this destructive insect pest and resistance problems through synergistic actions. Moringa oleifera, often known as the 'miracle tree', has evolved into a plethora of secondary metabolites in the root system that possess exceptional insecticidal potency and interfere with the defensive mechanisms of other plant genera. The objective of this study was to examine the effects of flupyradifurone 17.09 % SL or afidopyropen 50 g/L DC alone and in combination with moringa root extract (MRE The formulation of *Spathoda campanulata* in a mother tincture form, as employed in homeopathy, offers the added advantage of being hydroethanolic and highly extractive, ensuring efficient solubilization of both polar and non-polar bioactives. This enhances its potential compatibility in aerosol or vaporizer systems, broadening its utility across indoor and peri-domestic applications. Another important consideration is the translation of these biochemical and chemical insights into real-world application formats. The use of the mother tincture in a vaporizer formulation, as part of a polyherbal blend, leverages the volatile nature of its active compounds while minimizing the need for synthetic solvents or carriers.[37] The consistency of bioactivity across biochemical assays and repellent efficacy in live cage studies confirms the functional integrity of *Spathoda campanulata* Q when incorporated into a formulation matrix. Moreover, the observed physical stability over 30 days further validates its suitability for commercial development, especially when targeting community-level vector control where product consistency and shelf life are essential.[38]

From a phytomedicinal perspective, the results of this study resonate with the traditional use of *Spathoda campanulata* in ethnomedicine for treating parasitic and inflammatory conditions. Its known antimicrobial, antioxidant, and anti-inflammatory properties may also confer additional health benefits when used in ambient air-diffused formats such as vaporizers. While these properties were not directly explored in this study, their contribution to the overall safety and acceptability of the formulation cannot be overlooked.[39] The convergence of traditional knowledge, phytochemical analysis, and mechanistic assays positions *Spathoda campanulata* Q as a promising agent in the evolving field of eco-friendly biopesticides. In synthesizing the chemical and biochemical findings of the study, it becomes evident that the larvicidal and repellent effects of *Spathoda campanulata* are not attributable to a singular mode of action. Rather, they arise from a combination of factors: chemical constituents interfering with neural and metabolic pathways, volatile compounds creating olfactory barriers, and enzyme inhibition compromising larval survival.[40] These layers of activity, when interpreted alongside the GC–MS data and enzyme inhibition profiles, underscore the botanical extract's potential as a multifaceted insect control agent. Importantly, this aligns with current trends in green chemistry and sustainable agriculture, where plant-based biocontrol strategies are increasingly favored over traditional chemical pesticides.

Conclusion

The findings of this study establish *Spathoda campanulata* Q as a promising botanical agent with significant larvicidal and repellent properties against *Aedes aegypti* and *Anopheles stephensi*. Its phytochemical richness, confirmed through GC–MS, TLC, UV, FTIR, and physicochemical profiling, revealed bioactive constituents with known neurotoxic and growth-disrupting effects. The mother tincture exhibited potent enzyme inhibition activity against AChE and GST, providing a mechanistic basis for its observed larvicidal efficacy, which was found comparable to the standard agent, Azadirachtin. Furthermore, incorporation of *Spathoda* into a polyherbal vaporizer enhanced its repellent action significantly, demonstrating both rapid knockdown and prolonged protection in cage studies. The stable formulation, combined with its multipronged mechanism of action, supports the use of *Spathoda campanulata* Q as an eco-friendly, safe, and effective component of integrated mosquito management strategies.

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