

## Integrated phytochemical analysis and biological evaluation of *Spathodea campanulata* bud fluid for eco- friendly mosquito control applications

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### Abstract

Mosquito- borne diseases remain a significant global public health challenge, necessitating the development of safe, eco-friendly, and effective alternatives to synthetic chemical repellents. The present study focuses on the formulation and evaluation of a herbal mosquito repellent cone using *Spathodea campanulata* calyx (bud) fluid and flower powder. Preliminary phytochemical screening of the calyx fluid revealed the presence of bioactive compounds such as flavonoids, carbohydrates, and anthraquinones, which are known for their insecticidal and larvicidal properties. Further characterization using FTIR spectroscopy confirmed the presence of functional group such as hydroxyl and amine groups, indicating phenolic and nitrogen- containing compounds. GCMS analysis identified 28 phytoconstituents, including alkanes, terpenoids, phenolics, esters, and aromatic compounds, many of which are associated with mosquito repellent and antimicrobial activities. The herbal cone was formulated using natural ingredients such as African tulip tree flower, bud fluid, champak, frangipani, linseed, cinnamon, charcoal and castor oil to ensure sustained combustion and slow release of active compounds. The prepared cones were evaluated for physical appearance, burning time, smoke density, ash content, and safety parameters. The formulation exhibited efficient burning with minimal smoke and demonstrated significant mosquito repellency and larvicidal activity. Collectively, the study highlights the potential of *Spathodea campanulata* as a novel sustainable, and plant- based alternative for mosquito control. The developed herbal cone offers an eco- friendly approach that could reduce dependence on synthetic repellents and contribute to public health protection.

**Keywords:** Phytochemical Analysis; Larvicidal activity; Eco- friendly formulation; Herbal mosquito repellent

### 1. Introduction

Mosquito-borne diseases continue to be a serious public health problem worldwide, contributing to nearly 17% of all infectious diseases and causing around 700,000 deaths each year. In India, the burden is particularly high, with over 1.5 million reported dengue cases and several thousand chikungunya infections in recent years. States like Karnataka have recorded a significant share of these cases. On a global scale, millions of people are affected annually, making mosquito control an urgent priority in both developing and developed regions. Mosquitoes belonging to the family *Culicidae* include more than 3,600 species, but only a few genera—*Aedes*, *Culex*, and *Anopheles*—are responsible for transmitting major human diseases. These vectors spread a wide range of pathogens, including viruses and parasites, leading to illnesses such as malaria, dengue, chikungunya, yellow fever, Japanese encephalitis, lymphatic filariasis, West Nile fever, and Zika. Among these, malaria remains one of the most severe, with hundreds of millions of cases reported annually and a high mortality rate, especially among children under five years of age. Dengue, transmitted mainly by *Aedes* mosquitoes, has also emerged as a rapidly spreading disease, placing billions of people at risk globally.

*Spathodea campanulata*, commonly known as the African tulip tree, is a tropical plant belonging to the family Bignoniaceae. It is widely distributed in many parts of Africa and Asia and is easily recognized by its bright reddish-

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orange flowers. One of the unique features of this plant is its flower buds, which contain a watery fluid within the calyx. This fluid has ecological importance, as it serves as a source of moisture for birds and other organisms, particularly during dry periods. Traditionally, different parts of *Spathodea campanulata* have been used in the treatment of various health conditions, including inflammation, pain, and metabolic disorders. Phytochemical investigations have shown that the plant contains several important secondary metabolites such as flavonoids, tannins, alkaloids, glycosides, and sterols. These compounds are known to contribute to its biological activities. The calyx fluid, in particular, has attracted attention due to its unique composition. Studies using GC-MS analysis have identified a variety of chemical constituents, including terpenoids, alkanes, esters, alcohols, and phenolic compounds, many of which may play a role in repelling insects.

Recent research suggests that extracts obtained from the leaves and flower buds of *S. campanulata* exhibit larvicidal and mosquito-repellent activity. The presence of volatile compounds further supports its potential use as a natural repellent. At the same time, the plant's unique physiological features, such as the presence of phytohormones in the calyx fluid, add to its scientific significance. In recent years, people have shown increasing preference for herbal mosquito repellents due to concerns about the safety of chemical-based products. Many commercially available repellents are associated with side effects such as skin irritation, unpleasant odor, and discomfort caused by smoke. This has created a demand for alternatives that are not only effective but also safer for regular use.

In this context, the present study focuses on the formulation and evaluation of an herbal mosquito repellent cone using *Spathodea campanulata* calyx fluid and flowers. The formulation is designed to provide a slow-release effect during combustion, allowing prolonged protection against mosquitoes. By using natural ingredients, the developed product aims to serve as a cost-effective and environmentally friendly alternative to conventional chemical repellents. Collectively, this study attempts to explore the potential of a plant-based approach for mosquito control, which may help reduce dependence on synthetic chemicals and contribute to safer public health practices.

To date, there are limited reports on the use of *S. campanulata* as a mosquitocidal or vector control agent. Therefore, this study represents a novel attempt to develop plant-based larvicidal and repellent formulations for sustainable mosquito control.

## 2. Materials and methods

### 2.1. Sample collection

Fresh flowers and buds of *Spathodea campanulata* were collected from an African tulip tree at different locations in Tiruppur, Tamilnadu. The healthy flowers and buds were selected and grouped into small, medium, and large based on the size of the intact flower buds.

### 2.2. Preparation of bud fluid and flowers

The buds were washed with distilled water to remove debris and surface contaminants. The calyx/bud fluid was extracted by gently pressing the bud to obtain the fluid and filtering it through Whatman No. 1 filter paper. The filtrate was stored in the refrigerator.

The flowers were shade-dried for 1-2 months and the dried flowers were fine powder.

### 2.3. Qualitative evaluation of phytochemicals

The calyx fluid of *S. campanulata* was taken and phytochemical screening was done to check the phytoconstituents present using standard reagents (Junaid R Shaikh et al., 2020). The following qualitative tests for the metabolites were done as follows:

### 2.4. Phytochemical characterization

- GC-MS analysis

Hydro-distilled essential oils were analyzed using a Clarus 600 GC-MS system (PerkinElmer, USA) at DRDO-Bharathiar University, Coimbatore. Separation was carried out on an Elite-5 MS column using helium as the carrier gas at a constant flow rate of 1 mL/min. A 1 µL sample was injected, and the oven temperature was programmed from 60 °C to 300 °C at a rate of 10 °C/min. The mass spectrometer operated under electron impact ionization at 70 eV with a scan range of 40–

600 Da. The obtained spectra were compared with the NIST (2008) library for compound identification (Vignesh et al., 2025).

## 2.5. Identification of components

Interpretation on GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST), having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr Dukes, 2013).

## 2.6. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule that could frequently be obtained from its absorption spectrum. A small quantity of the *S. campanulata* extract was mixed with dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. The FTIR spectrum of the sample was recorded in the range 4000–400 cm<sup>-1</sup>. The spectrum obtained was analyzed to determine the functional groups present in the extract. Peaks were compared with standard FTIR reference data for identification (P.K Jain *et al.*, 2016).

## 2.7. Larvicidal bioassay test

In order to determine the efficacy of *S. campanulata* calyx fluid in various concentration were prepared. The concentrations tested included 25%, 50%, 75% and 100% of the extract. To ensure consistency and accuracy, each concentration was placed in a separate flask. Calyx extract on larvae were tested with three replications and positive and negative control were maintained. (Abdul Ahad et al., 2024 & Anitha Rajasekaran and Gheethapriya Duraikannan, 2012))

- Positive control: 1ml neem oil + 100 ml water (standard)
- Negative control: 100 ml water

The next step of the experiment involved Third and fourth instar larvae were selected and 20 larvae were introduced to each concentration. During the testing period, precise measurements and observations were taken to assess the impact of the different concentrations on the larvae. The number of tested larvae, the number of dead larvae, the mean number of dead larvae, mean percentage and the duration of exposure were analyzed. The mortality rate was observed after 24 hours and the percentage mortality was calculated.

The following formula is used to determine the mortality percentage:

Percentage of mortality % = Mean number of dead larvae / Number of tested larvae x 100

## 2.8. Statistical Analysis

The statistical tools that were used in this study are the following: the mean number of dead of mosquito larvae and percentage of larval mortality was calculated. The efficacy of each concentration was analyzed by chi-square.

Formulation of an herbal mosquito repellent cone

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## 3. Materials

The present study utilized herbal and natural ingredients known for their insect-repellent and combustion-supporting properties. The materials included (Table: 1)

**Table 1** Formulation contains calyx fluid with ingredients

| S. No | Ingredients                    | Quantity | Role                                           |
|-------|--------------------------------|----------|------------------------------------------------|
| 1.    | African tulip tree flower      | 3 gm     | Insecticide, mosquito repellent, Anti-malarial |
| 2.    | Champak                        | 1 gm     | Fragrance                                      |
| 3.    | Frangipani                     | 1 gm     | Fragrance                                      |
| 4.    | Linseed                        | 1 gm     | Binder and slow, steady burning                |
| 5.    | Cinnamon                       | 0.5 gm   | Aromatic and Anti-microbial                    |
| 6.    | Charcoal                       | 0.2 gm   | Combustion                                     |
| 7.    | Castor oil                     | 1ml      | Binder                                         |
| 8.    | African tulip tree calyx fluid | q.s      | Anti-microbial                                 |

### 3.1. Preparation of Herbal Powders:

#### 3.1.1. Procedure

All powdered ingredients used in the mosquito repellent cone formulation, namely African tulip tree (*Spathodia campanulata*), champak (*Magnolia champaka*), Frangipani (*Plumeria rubra*), Linseed (*Linum usitatissimum*), Cinnamon, Castor oil, and charcoal were prepared using standard methods to ensure quality and uniformity. Plant-based materials such as African tulip tree, Champak, and Frangipani were selected fresh, washed thoroughly with clean water to remove dirt and impurities, and dried in a shaded, well-ventilated area for several days until completely moisture-free. The dried materials were then ground into a fine powder and passed through a sieve to obtain a uniform particle size. Dry commercial ingredients like charcoal were used directly and similarly passed through a sieve to ensure a consistent texture. Castor oil was used directly in liquid form during the formulation process without any prior drying or modification. All prepared powders were stored in airtight containers in a cool, dry environment to preserve their functional properties.

#### 3.1.2. Preparation of mosquito repellent cone

The preparation process began with blending the specified quantities of dry powders, namely champak, frangipani, African tulip tree flower, cinnamon, linseed and charcoal in a clean dry mortar. The powders were thoroughly homogenized to ensure a uniform mixture. Subsequently, measured amounts of castor oil were gradually incorporated to facilitate the even distribution of constituents. Calyx fluid was then added incrementally to achieve a cohesive. The semi-solid mass was moulded into the shapes of cones and these cones were prepared by mould. The cones were demoulded and stored in airtight, moisture-resistant containers at ambient temperature to preserve their bioactive properties and maintain formulation stability for further evaluation.

The evaluation of the herbal mosquito repellent cone involves several aspects, including efficacy, physical properties, user safety, and practicality. The following parameters are critical in assessing the performance and suitability of the herbal mosquito repellent cone as an alternative to conventional chemical-based mosquito repellents (Appala Anjalietal.,2025).

#### 3.1.3. Physical Appearance

The prepared herbal mosquito repellent cone was evaluated for their colour, Odour, size, shape, texture, homogeneity, uniformity and physical state. These observations were made through direct visual and olfactory inspection.

#### 3.1.4. Flammability and Burning Time

To assess flammability a cone was ignited and observed for flame propagation and smoke production. The burning time recorded until the cone was fully consumed was found to be approximately 24 minutes indicating efficient combustion with minimal smoke. Variations in burning time are influenced by cone length, diameter and formulation quality.

### 3.1.5. Ash Value

The completely burned residue of the cones was collected and weighed to determine the ash content which reflects the inorganic residue post-combustion.

### 3.1.6. Smoke Toxicity Test

To test for smoke toxicity mosquitoes were attracted into a chamber using worn socks and then exposed to the smoke of a burning cones for 30 minutes. Mortality was recorded at 15-30 minutes intervals to assess the lethal impact of the smoke.

### 3.1.7. Smoke Visibility Test

The density and visibility of smoke from the cones were evaluated visually upon ignition. Observations were made on smoke spread thickness and irritation.

### 3.1.8. Irritability test

This test is used to determine whether the prepared dhoop cone irritates skin in any way.

### 3.1.9. Mosquito repellency test

The repellent activity was evaluated in situ by placing the cones in mosquito-prone environments during evening and night. Locations included laboratory corners, near shrubs, and public areas like the cafeteria. Observations were made on mosquito presence before and after burning, and public feedback was documented.

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## 4. Result and discussion

### 4.1. Phytochemical Screening

The preliminary phytochemical analysis of the calyx fluid of *Spathodea campanulata* revealed the presence of carbohydrates, flavonoids, and anthraquinones. Flavonoids are widely recognized for their ability to interfere with mosquito larval development and exhibit toxic effects against various mosquito species. They also possess strong antioxidant, antimicrobial, and insecticidal properties (Gani et al., 2024). Anthraquinones are known to act as natural repellents and feeding deterrents, making them useful in controlling insect pests (Krishnan Pathy, 2021).

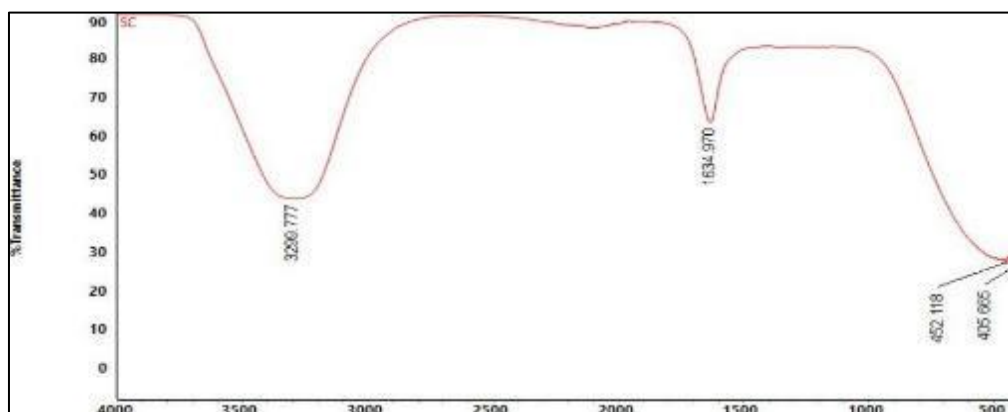
Earlier studies on *S. campanulata* have mainly focused on other plant parts such as leaves, bark, and flowers. Phytochemical screening of these parts has report the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, glycosides, and saponins in different extracts (Abdullahi et al., 2017; Naqab et al., 2018). These compounds are often associated with significant biological activities, including antimicrobial, antimalarial, antioxidant, and anti-inflammatory effects. For example, flavonoids and phenolic compounds contribute to antioxidant activity, while alkaloids and terpenoids are linked to anti-plasmodial and insecticidal properties (Gani et al., 2024; Geevarghese et al., 2024).

In addition, studies on flower and leaf extracts have reported the presence of tannins, glycosides, and phenolic compounds, which enhance the medicinal value of the plant. Methanolic and ethyl acetate extracts have further demonstrated a wide range of phytoconstituents, including proteins, amino acids, reducing sugars, and secondary metabolites (Abin et al., 2024). These findings highlight the importance of phytochemical composition in determining the biological potential of plant-based materials. However, despite several reports on different parts of *S. campanulata*, there is very limited information available on the phytochemical composition of its calyx fluid. Therefore, the present study provides new insights into this less explored plant component. The bioactive compounds identified in the calyx fluid may be responsible for its observed larvicidal and mosquito repellent activity, supporting its potential as a natural alternative for mosquito control.

### 4.2. Fourier Transform infrared (FTIR) spectroscopy of *S. campanulata* calyx fluid

The Fourier Transform Infrared (FTIR) spectroscopy was carried out to identify the major functional groups present in the calyx fluid extract of *Spathodea campanulata*. The FTIR spectrum showed (Figure 1) a broad peak at  $3299.777\text{ cm}^{-1}$  corresponding to O-H stretching vibrations indicating the presence of alcohols and phenolic compounds. The absorption band at  $1634.970\text{ cm}^{-1}$  observed in the FTIR spectrum was assigned to N-H bending vibrations, suggesting the presence of primary amines or nitrogen-containing compounds. The peaks at  $405.655$  and  $452.118\text{ cm}^{-1}$  in FTIR

indicate the low-frequency region and remain unassigned, possibly representing complex skeletal vibrations of secondary metabolites.



**Figure 1** FTIR of *S. campanulata* calyx fluid

Similar observations have been previously reported for *Spathodea campanulata* bud fluid extracts using FTIR spectroscopy, which revealed the presence of various functional groups associated with different phytoconstituents. The vibrational frequencies were assigned to specific functional groups. The calyx fluid of *S. campanulata* exhibited characteristic bands at  $1641.42\text{ cm}^{-1}$  and  $3388.93\text{ cm}^{-1}$ , indicating the presence of carbonyl (C=O) and hydroxyl (–OH) groups (Naganagouda Goudar et al., 2020). The presence of phenolic (O–H), carbonyl (C=O), and hydroxyl (–OH) functional groups suggests that the plant extract contains bioactive compounds capable of interfering with the larval nervous and respiratory systems. Phenolic compounds and flavonoids are well known for their antioxidant, antimicrobial, and larvicidal properties. Recent studies have reported that phenolic-rich extracts exhibit strong mosquito larvicidal activity due to their ability to disrupt the digestive and respiratory physiology of larvae (Kumar et al., 2021). A similar report also confirmed the presence of characteristic bands at  $1641.42\text{ cm}^{-1}$  and  $3388.93\text{ cm}^{-1}$  in the calyx fluid of *S. campanulata*, corresponding to carbonyl and hydroxyl groups (Komal Chavan et al., 2022).

#### 4.3. Gas Chromatography–Mass Spectroscopy (GC–MS)

Gas Chromatography–Mass Spectrometry (GC–MS) is one of the most effective techniques for identifying chemical constituents, including long-chain and branched hydrocarbons, alcohols, acids, and esters. The chemical composition of the *Spathodea campanulata* calyx fluid was analyzed using GC–MS, which revealed the presence of 28 compounds. The identification of these phytochemical constituents was based on their retention time (RT), molecular formula, molecular weight (MW), and peak area percentage, as presented in Figure 2.

The identified compounds include glyceraldehyde, dihydroxyacetone, 1,2-cyclopentanedione, propanoic acid (3-hydroxy-, methyl ester), 3(2H)-furanone (4-hydroxy-5-methyl-), thymine, butanedioic acid (monomethyl ester), 2-furanone (3,4-dihydroxytetrahydro-), benzaldehyde (3-benzyloxy-2-fluoro-4-methoxy-), 1,2,3-propanetriol (1-acetate), heptanoic acid (6-oxo-), ethanone [1-(2-hydroxy-5-methylphenyl)-], butanoic acid [4-amino-3-hydroxy-(±)-], 4-vinylbenzene-1,2-diol, cyclohexaneethanol (2-methylene-), 1,4-naphthalenediol, 3-O-benzyl-D-glucose, D-glycero-D-ido-heptose, 4,6-octadienoic acid, 3-buten-2-ol (2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-), 1-hydroxybicyclo[2.2.2]oct-5-en-2-yl methyl ketone, benzenepropanoic acid [3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester], 9,12-octadecadienoic acid (Z,Z)-, 1-heptanol (2-propyl-), hexadecanoic acid [2-hydroxy-1-(hydroxymethyl)ethyl ester], ethyl(1-adamantylamino) carbothioylcarbamate, and related derivatives.

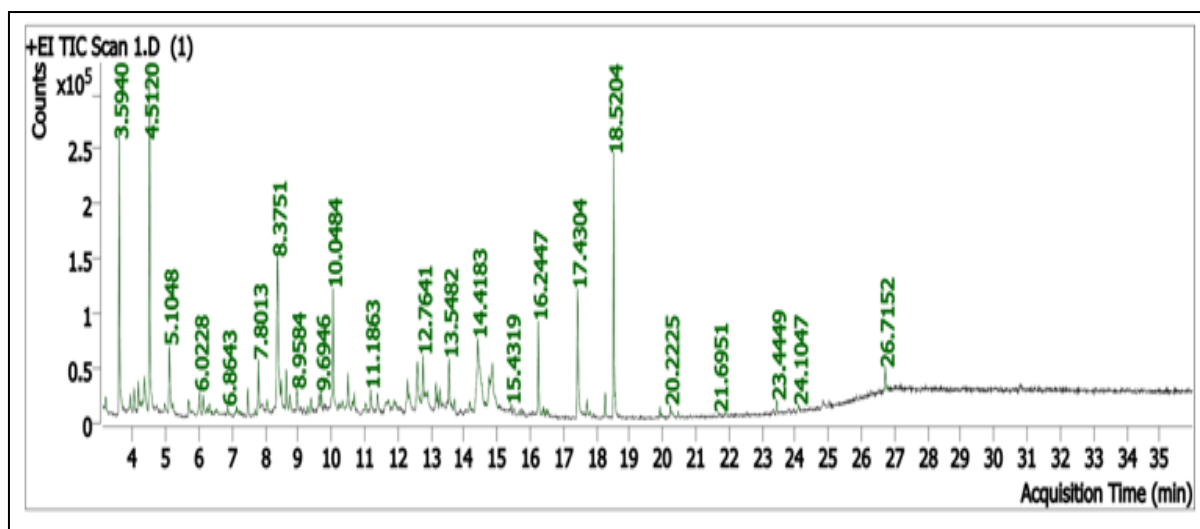


Figure 2 GC-MS of *S. campanulata* calyx fluid

The GC-MS analysis of *Spathodea campanulata* calyx fluid revealed the presence of several bioactive compounds, including 1,3,3-trimethoxybutane (3.91%), 1,3-dioxolane-4-methanol, 2-ethyl (6.44%), isopropyl myristate (3.88%), malathion (4.03%), and dimethyl-substituted alkanes. These compounds are known to exhibit biological activities such as anti-inflammatory, antimicrobial, and antioxidant effects (Hachlafi et al., 2023). Notably, isopropyl myristate is widely used as a skin penetration enhancer, supporting its relevance in topical therapeutic applications (Masakhanian et al., 2004). Previous studies on *S. campanulata* and other plant species have reported the presence of terpenoids, fatty acids, phenolics, and hydrocarbons, which are strongly associated with larvicidal and insecticidal activities (Pathalam Ganesan et al., 2023). Compounds such as phenylpropanoids and long-chain fatty acids have been shown to contribute significantly to larval mortality. Earlier reports on *S. campanulata* also indicate considerable chemical diversity, including benzyl alcohol, eugenol, thymol, and n-hexadecanoic acid, which are linked to various pharmacological properties. In the present study, the identified compounds mainly belong to alkanes, terpenoids, aromatic hydrocarbons, alcohols, esters, carbonyl compounds, and phenolics. These phytochemical classes are well known for their larvicidal and repellent activities. FTIR analysis further confirmed the presence of functional groups corresponding to these compounds.

#### 4.4. Larvicidal activity

In the present study, we aimed to evaluate whether the calyx fluid of *Spathodea campanulata* could be used for mosquito control. A clear response was observed in all immature stages of mosquitoes exposed to the bud extract. The larvicidal activity of *S. campanulata* was assessed against third and fourth instar larvae using different concentrations (25%–100%), as presented in Table 2.

Table 2 Larvicidal activity of *S. campanulata* calyx fluid at different concentrations

| Concentration | Total no. of larvae | I  | II | III | Sum | Mortality % | Duration |
|---------------|---------------------|----|----|-----|-----|-------------|----------|
| 25%           | 60                  | 7  | 7  | 8   | 22  | 37%         | 24hrs    |
| 50%           | 60                  | 12 | 13 | 13  | 38  | 63%         | 24hrs    |
| 75%           | 60                  | 17 | 16 | 17  | 50  | 83%         | 24hrs    |
| 100%          | 60                  | 20 | 20 | 20  | 60  | 100%        | 24hrs    |

The larvicidal activity of *Spathodea campanulata* bud extract showed a clear concentration-dependent effect. At 100% concentration, complete larval mortality (100%) was observed within 24 hours, indicating strong activity. At 75%, 83% mortality was recorded, while 50% and 25% concentrations resulted in 63% and 37% mortality, respectively. Although the effectiveness decreased with dilution, the extract still maintained noticeable activity even at lower concentrations. These results suggest a rapid mode of action and confirm the strong larvicidal potential of the bud extract. In addition, the extract exhibited significant mosquito repellent activity, with protection efficiency increasing along with

concentration and reaching maximum levels at higher doses. The observed biological activity may be due to the presence of phytochemicals such as flavonoids, carbohydrates, and anthraquinones, which are known to act either individually or synergistically to affect mosquito larvae.

The present findings are in agreement with earlier reports on the larvicidal activity of *S. campanulata* and other plant species. Previous studies have demonstrated that leaf extracts of *S. campanulata* possess significant activity against mosquito vectors such as *Aedes aegypti* and *Anopheles stephensi* (Pravin and Mohanraj, 2019). Similarly, plant extracts from species like *Leucas aspera*, *Delonix elata*, *Erythrina indica*, and *Lippia adoensis* have also shown effective larvicidal properties. The presence of bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids in *S. campanulata* has been previously linked to insecticidal and antimalarial activities (Juliet et al., 2012). In line with these reports, the present study confirms that the calyx fluid of *S. campanulata* possesses strong larvicidal and repellent properties, highlighting its potential as a natural and eco-friendly agent for mosquito control.

#### 4.5. Formulation and evaluation of mosquito repellent cone

Mosquito repellent cones were prepared using *Spathodea campanulata* flower and calyx fluid along with herbal ingredients such as champak, frangipani, linseed, cinnamon, and castor oil. The formulated cones showed effective mosquito repellency without any noticeable side effects. They burned steadily with moderate smoke and produced less irritation compared to synthetic coils. Unlike chemical-based products containing allethrin or other pyrethroids, the herbal cones did not cause eye or respiratory discomfort, making them more suitable for regular use. The repellency observed may be attributed to the presence of bioactive compounds in the ingredients. For example, cinnamon contains cinnamaldehyde and eugenol, which are known for their fumigant and repellent action (Bhowmick et al., 2019), while champak and frangipani are rich in sesquiterpenes that contribute to mosquito repellent activity (Rajkumar et al., 2021). Linseed and castor oil act as natural binders and help in the slow release of volatile compounds, thereby prolonging the duration of protection.

Similar studies using plant-based materials such as *Azadirachta indica*, *Ocimum sanctum*, and *Cinnamomum verum* have also reported good mosquito repellency with minimal toxicity (Valarmathi et al., 2022; Chavhan et al., 2022). However, there are only limited reports on the use of *S. campanulata* flower and calyx fluid in such formulations. In the present study, the developed cone showed comparable or improved repellency, which may be due to the combined action of phenolics, terpenoids, and other phytochemicals identified through analytical studies. The mixture of these compounds likely enhances the repellent effect through synergistic action and sustained release. Overall, the findings suggest that *S. campanulata*-based mosquito repellent cones can serve as a safe, eco-friendly, and effective alternative to synthetic repellents.

#### 4.6. Evaluation tests

##### 4.6.1. Physical Appearance

The physical characteristics of the mosquito repellent cones were evaluated based on parameters such as colour, odour, size, shape, surface texture, and homogeneity, as presented in Table 4. The formulation exhibited a uniform cone shape with a standard size of 4.5 cm. The cones showed a smooth surface texture and good homogeneity, indicating proper blending and compatibility of the herbal ingredients and binders. The colour of the cones ranged from black to brown, and they possessed a characteristic pleasant herbal odour, enhancing their acceptability as natural mosquito repellents.

**Table 3** Physical appearance of formulation

| S. No | Physical appearance parameters | Herbal dhoop cone     |
|-------|--------------------------------|-----------------------|
| 1.    | Colour                         | Brown with black      |
| 2.    | Odour                          | Pleasant herbal smell |
| 3.    | Size                           | 4.5 cm                |
| 4.    | Shape                          | Conical               |
| 5.    | Surface texture                | Smooth                |
| 6.    | Homogeneity                    | Homogenous            |

#### 4.6.2. Burning time

Burning time is a critical parameter influencing repellent efficacy. The formulation exhibited a maximum burning duration of 35 minutes. This prolonged burning time may be attributed to the binder concentration, herbal composition, and compactness of the cone. A longer burning duration is desirable for sustained repellent action, and the formulation performed optimally in this regard.

#### 4.6.3. Ash Content

Ash values indicate the efficiency of combustion, with higher ash content suggesting the presence of denser or mineral-rich organic components, as shown in Table 4. The formulation exhibited a higher ash residue, likely due to the increased proportion of such constituents. However, the observed values are within acceptable limits for herbal incense products.

#### 4.6.4. Smoke Visibility

Smoke visibility was assessed visually, as presented in Table 4. The formulation exhibited moderate smoke visibility, which may contribute to enhanced mosquito repellency, as smoke acts as a deterrent. However, it is important to maintain a balance, as excessive smoke may cause user discomfort.

#### 4.6.5. Mosquito Repellent Activity

The repellent activity was evaluated in mosquito-prone environments such as bedrooms, classrooms, and washrooms. In all test areas and at different time intervals, mosquitoes were observed to move away upon exposure to the smoke. No adverse effects, such as irritation, coughing, tearing, or headache, were reported, as shown in Table 4. These observations confirm the effectiveness of the herbal repellent cone as a non-toxic and environmentally friendly alternative to conventional mosquito repellents.

**Table 4** Evaluation parameters of mosquito repellent cone formulation

| S. No | Parameters          | Observation |
|-------|---------------------|-------------|
| 1.    | Burning time        | 30 min      |
| 2.    | Ash value           | 0.829gm     |
| 3     | Smoke toxicity test | No toxicity |
| 4.    | Smoke visibility    | Average     |
| 5.    | Irritability test   | No          |
| 6.    | Repellent activity  | Effective   |

The present study on *Spathodea campanulata* bud fluid revealed its rich phytochemical composition and promising potential for mosquito control through combined analytical and biological evaluation. Qualitative screening confirmed the presence of flavonoids, carbohydrates, and anthraquinones, which are known to exhibit insecticidal, larvicidal, and repellent activities. FTIR analysis supported these findings by indicating functional groups such as phenols and alcohols, suggesting the presence of active biomolecules. Further, GC-MS analysis identified a range of volatile and semi-volatile compounds, including terpenoids, phenolics, and fatty acid esters, which may contribute to larval toxicity and enhanced biological activity.

The larvicidal assay showed a clear concentration-dependent response, with complete mortality observed at higher concentrations, indicating strong efficacy of the bud fluid. In addition, the herbal mosquito repellent cone formulated using the bud fluid along with natural ingredients such as cinnamon, frangipani, champak, linseed, and castor oil demonstrated good burning properties and effective mosquito repellency. The presence of aromatic compounds, particularly from cinnamon, likely enhanced the repellent action. Overall, the results suggest that *S. campanulata* bud fluid, supported by phytochemical and instrumental analysis, has strong potential as a natural, eco-friendly alternative for mosquito vector control.

## 5. Conclusion

The present study was carried out to evaluate the mosquito repellent potential of *Spathodea campanulata* bud fluid using phytochemical, analytical, and formulation approaches. The results clearly indicate that the bud fluid possesses considerable potential as a natural mosquito control agent. Preliminary phytochemical screening confirmed the presence of important secondary metabolites such as flavonoids, carbohydrates, and anthraquinones, which are known for their larvicidal, insecticidal, and repellent activities. FTIR analysis supported these findings by identifying functional groups like hydroxyl, carbonyl, and amine groups, suggesting the presence of biologically active compounds. GC-MS analysis further revealed various bioactive constituents, including terpenoids, phenolics, and fatty acid derivatives, which are commonly associated with mosquito control activity. The larvicidal study showed a clear concentration-dependent effect, with complete mortality observed at higher concentrations within 24 hours, indicating strong and rapid action. Proper control results further confirmed the reliability of the experiment. In addition, the herbal mosquito repellent cone prepared using *S. campanulata* along with natural ingredients such as *Magnolia champaca*, *Plumeria rubra*, *Linum usitatissimum*, and *Cinnamomum verum* showed good physical properties, steady burning, and effective repellency. The formulation produced less irritating smoke and did not cause discomfort, making it safer for domestic use. Although synthetic products showed quicker initial action, the herbal formulation provided longer-lasting protection. Overall, the study highlights that *S. campanulata*-based formulations can serve as a safe, eco-friendly, and sustainable alternative for mosquito control, though further studies are needed for large-scale application and standardization.

## Compliance with ethical standards

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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