

## ***Pennisetum setaceum* extracts and copper nanoparticles: A potential solution for human pathogens and mosquito-borne diseases**

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### **Abstract**

The study investigates the green synthesis of copper nanoparticles using *Pennisetum setaceum* extract, highlighting their remarkable Mosquito larvicidal activity against *Culex quinquefasciatus* larvae. The nanotechnology integration enhances bioavailability and cellular penetration in *C. quinquefasciatus* larvae, boosting effectiveness at lower concentrations. Copper nanoparticles (CuNPs) achieved 100% mortality at  $\geq 100$  ppm after 24 hours. Ethyl acetate extract caused  $58.87\% \pm 1.56\%$  mortality at 150 ppm, while ethanol extract showed  $64.90\% \pm 1.73\%$  mortality at 120 ppm. At 1000 ppm, CuNPs completely prevented hatching (0%), ethyl acetate extract reduced it to 15.72%. Phytochemical screening of *Pennisetum setaceum* extracts revealed the presence of tannins, saponins, flavonoids, and phenols in both ethyl acetate and ethanol extracts, with cardiac glycosides detected only in the ethanol extract. GC-MS analysis of the ethanol extract identified neophytadiene (32.05%), benzenepropanoic acid (55.21%), and bis(2-ethylhexyl) phthalate (12.74%) as major compounds. Ethanol extract and copper nanoparticles (CuNPs) demonstrated significant antimicrobial activity against various pathogens, with CuNPs showing the highest efficacy (inhibition zones up to  $21.3 \pm 1.5$  mm against *V. cholerae* and *Aspergillus niger*), MIC values of 55.8-81% for ethanol extract and 69.2-82.9% for CuNPs, and MBC values of 187.5-750  $\mu\text{g/ml}$  for ethanol extract with *E. coli* being most susceptible at 375  $\mu\text{g/ml}$ . Toxicity tests using *Artemia salina* indicated that *P. setaceum* extracts were less toxic than potassium dichromate, with  $\text{LC}_{50}$  values of 801.23ppm for CuNPs and 730.81 ppm for ethanol extract. These findings suggest that *P. setaceum*, a common weed, holds potential for medicinal applications and mosquito larvicidal purposes and warrants further investigation into pest management approaches.

**Keywords:** *Pennisetum setaceum*, Larvicidal activity, phytochemicals, antimicrobial activity, toxicity

### **Introduction**

The increasing prevalence of antibiotic-resistant pathogens has sparked significant interest in developing new, effective antimicrobial agents (Drakhshaan *et al.*, 2025 <sup>[10]</sup>; Breijyeh *et al.*, 2023) <sup>[6]</sup>. In this context, plant extracts, particularly those from invasive species like *P. setaceum*, have garnered attention for their diverse phytochemical composition and potential therapeutic applications (McGaw *et al.*, 2021) <sup>[19]</sup>. *P. setaceum*, commonly known as fountain grass, is an invasive species causing ecological and economic issues globally (Zhang *et al.*, 2024) <sup>[33]</sup>. However, its potential medicinal properties, including antimicrobial and phytochemical attributes, have been explored (Hardy *et al.*, 2025) <sup>[15]</sup>. Recent studies have highlighted the promise of nanotechnology, especially the use of copper nanoparticles, in enhancing the antimicrobial properties of natural compounds (Jayachandran *et al.*, 2021) <sup>[17]</sup>. Copper nanoparticles exhibit strong antimicrobial activity against a wide range of pathogens, making them a valuable tool for developing new antimicrobial agents (Asghar & Asghar, 2020) <sup>[2]</sup>. Moreover, the integration of nanotechnology has enhanced the mosquito larvicidal properties of natural compounds, particularly against *Culex quinquefasciatus* (Gowthami *et al.*, 2023) <sup>[13]</sup>.

This study investigates the antimicrobial potential of *P.*

*setaceum*, emphasizing its diverse phytochemical composition. Through Gas Chromatography-Mass Spectrometry (GC-MS), we aim to identify and characterize bioactive compounds responsible for its antimicrobial efficacy (Modi *et al.*, 2025) <sup>[20]</sup>. The study focuses on the antimicrobial activity of *P. setaceum* extracts against a panel of clinically relevant microorganisms, including *Vibrio cholera*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, and *Escherichia coli*. The findings of this research will provide valuable insights into the therapeutic applications of *Pennisetum setaceum* and contribute to the growing body of knowledge on weed-derived antimicrobial agents (Windarsih *et al.*, 2025) <sup>[32]</sup>. Transforming invasive weeds into valuable medicinal resources represents an innovative and sustainable approach to addressing health-related challenges (McGaw *et al.*, 2021) <sup>[19]</sup>. Ultimately, this study aims to contribute to the development of new, effective antimicrobial agents and offer new insights into the potential therapeutic uses of this invasive weed.

### **Materials and Methods**

#### **Sample collection**

Fresh *Pennisetum setaceum* plant samples were collected from Kanyakumari district ( $8^{\circ}14'19.8''\text{N}$ ,  $77^{\circ}31'30.1''\text{E}$ ), Tamil Nadu.

### Preparation of extract

The powdered plant parts were soaked in each solvent for 48 hours to isolate the compounds. Bioactive compounds were extracted from 20 g of powdered plant material using 100 ml of ethanol and 100 ml of ethyl acetate solvents. (Modi *et al.*, 2025)<sup>[20]</sup>

### Copper Nanoparticle Synthesis

*Pennisetum setaceum* plant, 10 g of dry powdered plant sample was added to 300 ml of 1 mM Copper sulfate solution. The mixture was stirred continuously at room temperature for 3 days. The solution was then centrifuged at 10,000 rpm for 15 minutes to collect the nanoparticles. The nanoparticles were washed with distilled water, then dried at 60°C for 24 hours for further characterization. (Shaik *et al.*, 2025)<sup>[27]</sup>.

### Phytochemical Screening

A meticulous methodology was applied to identify and quantify the bioactive compounds in *Pennisetum setaceum*. This process encompassed tests for alkaloids, flavonoids, tannins, saponins, and other secondary metabolites renowned for their therapeutic potential. (Dejene *et al.*, 2025)<sup>[9]</sup>

### Spectroscopy analysis (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the chemical composition of extracts. This method helped identify and quantify volatile and semivolatile compounds, revealing the bioactive constituents responsible for anti-bacterial activity. (Abubakar *et al.*, 2025)<sup>[1]</sup>

### Mosquito (*Culex quinquefasciatus*) larvicidal activity

The larvicidal bioassay involved exposing third-instar larvae of *C. quinquefasciatus* (n=30 per concentration) to serial concentrations (30 ppm, 60 ppm, 90 ppm, 120 ppm, 150 ppm) of ethyl acetate, ethanol, and copper nanoparticles (CuNPs) for 24, 48, and 72 hours. The experiments were accurately conducted in triplicate to ensure the reproducibility and reliability of the results. The controlled environmental conditions were maintained at 27 ± 2°C temperature and 75 ± 5% relative humidity (RH) throughout the experiment. A 0.5% dimethyl sulfoxide (DMSO) solution served as a control to account for any potential effects of the solvent. Larval mortality was meticulously recorded at 24, 48, 72 hours post-exposure. The data collected were then subjected to statistical analysis to determine the lethal effects of the extracts at the various concentrations and exposure times. (Pavela *et al.*, 2019)<sup>[24]</sup>

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

### Egg hatchability of mosquitoes

The hatchability of *Culex quinquefasciatus* were studied using a controlled experimental setup. Freshly laid eggs (no older than 24 hours) were exposed to varying concentrations of ethanol, ethyl acetate, and CuNPs extracts derived from *P. setaceum*. These concentrations covered from 1 to 1000 ppm, specifically including 1, 10, 100, 200, 300, 500, 700, 900, and 1000 ppm, and were tested in triplicate. The experiments were conducted under standardized

environmental conditions of 27 ± 2°C temperature and 75 ± 5% relative humidity, with 0.5% DMSO used as a control. The hatchability of the eggs was assessed 72 hours after treatment. The outcomes were quantified as the percentage of eggs that successfully hatched. (Silva *et al.*, 2019)<sup>[28]</sup>

$$\text{Hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100 \%$$

### Antimicrobial activity, MIC, and MBC

The antimicrobial activity against bacteria such as *Vibrio cholera*, *Pseudomonas aeruginosa* and *Escherichia coli* and fungi *Candida albicans*, and *Aspergillus niger* were assessed using the well diffusion method. A 100 µL sample was loaded into each well. The diameter of the inhibition zones was measured in millimeters (mm) after 24 hours of incubation at 37°C, indicating the sample's potency. (Singh *et al.*, 2025)<sup>[30]</sup> The minimum inhibitory concentration (MIC) of the plant extract was determined using the microdilution method in a 96-well plate. Each well was prepared with 100 µL of nutrient broth. The plant extract was added to the first well at an initial concentration of 4000 µg/mL and serially diluted in subsequent wells to concentrations of 2000, 1000, 500, 250, and 125 µg/mL. A bacterial suspension (25 µL) with a concentration of 1 × 10<sup>7</sup> CFU/mL was added to each well. The plates were incubated at 37°C for 24 hours. After incubation, the MIC was identified as the lowest concentration of the plant extract that inhibited visible bacterial growth. The MIC was confirmed by measuring optical density (OD) at 600 nm using an ELISA reader immediately following visual inspection. Additionally, a 25-µL solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg MTT dissolved in 1 mL of sterile water) was added to each sample and incubated for 4 hours at 37°C to further assess cell viability. (Elshikh *et al.*, 2016)<sup>[11]</sup>

$$\text{Percentage of Inhibition} = \frac{\text{OD of Control} - \text{OD of Test}}{\text{OD of Control}} \times 100 \%$$

The minimum bactericidal concentration (MBC) of the plant extract was determined by subculturing from the wells where no visible growth was observed during the MIC assay. After the MIC determination, 10 µL from each well that showed no visible growth was subcultured onto nutrient agar plates. The agar plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for bacterial growth. The MBC was defined as the lowest concentration of the plant extract that resulted in no observable growth on the agar plates, indicating that the bacteria had been killed. This method ensures that the MBC accurately reflects the concentration at which the plant extract is bactericidal. (Syromyatnikov *et al.*, 2025)<sup>[31]</sup>

### Toxicity

For toxicity study, 30 *Artemia salina* per test were exposed to concentrations: 1, 10, 100, 500, 1000 µg/ml of ethyl Acetate and ethanol extracts. Potassium dichromate at 1000 µg/ml was used as the positive control. Mortality was recorded after 24 hours. (Parra *et al.*, 2001)<sup>[23]</sup>

### Statistical Analysis

Data analysis was performed using Microsoft Excel 2007 and SPSS 12 to conduct one-way ANOVA at the 95%

confidence interval. Significance was determined by P-values less than 0.05. LC<sub>50</sub>, LC<sub>90</sub>, and LC<sub>95</sub> values were derived using linear regression in R software.

## Result and Discussion

### Phytochemical Screening

The phytochemical screening of *Pennisetum setaceum* indicates the presence of several key compounds. Tannins, saponins, flavonoids, cardiac glycosides, and phenols were found in both ethyl acetate and ethanol extracts, suggesting potential antibacterial activity. However, alkaloids, steroids, quinones, and terpenoids were not detected in either solvent, indicating a lack of certain pharmacological activities. The phytochemical screening of *P. setaceum* identified tannins, saponins, flavonoids, cardiac glycosides, and phenols in both ethyl acetate and ethanol extracts. These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, and cardiovascular benefits. Compared to Ojo *et al.* (2022) [22], who found polyphenols, flavonoids, and tannins in Jordanian fountain grass leaves, the presence of saponins in *P. setaceum* suggests variability due to extraction methods or plant sources. Unlike Jack *et al.* (2020) [16], who detected alkaloids and steroids in *P. purpureum*, these compounds were absent in *P. setaceum*, highlighting phylogenetic differences. Consistent with Budiyantha *et al.* (2024) [7], ethanol extracts yielded higher phenolic content, crucial for antioxidant and anti-inflammatory activities. Solvent polarity significantly impacts extraction, revealing *P. setaceum*'s unique phytochemical profile. Phylogenetic divergence likely influences secondary metabolite profiles. Further research is needed to explore *P. setaceum*'s medicinal potential and factors affecting its secondary metabolite production, with implications for developing novel therapeutic agents.

**Table 1:** Phytochemical analysis of *Pennisetum setaceum* leaf extract

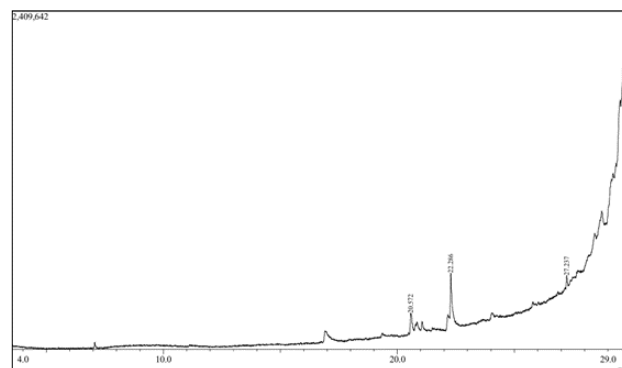
| Phytochemical test | Ethyl Acetate | Ethanol |
|--------------------|---------------|---------|
| Alkaloid           | -             | -       |
| Steroid            | -             | -       |
| Tannines           | +             | +       |
| Saponins           | +             | +       |
| Flavonoids         | +             | +       |
| Cardiac Glycosides | +             | -       |
| Quinone            | -             | -       |
| Trepeoid           | +             | -       |
| Phenols            | -             | +       |

'+' presence '-' absence

### GC-MS Analysis

The GC-MS analysis of the ethanol extract of *Pennisetum setaceum* identified three major compounds. Neophytadiene was the most abundant, representing 32.05% of the total area, followed by Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, ethyl ester at 55.21%, and Bis(2-ethylhexyl) phthalate at 12.74%. These findings suggest a chemical profile that could be associated with various biological activities, highlighting the plant's potential for further pharmaceutical exploration. The GC-MS analysis of *P. setaceum* identified key compounds like Neophytadiene, Benzenepropanoic acid, and Bis(2-ethylhexyl) phthalate, which have potential anti-

inflammatory, antimicrobial, and antioxidant properties. As reported by Braga *et al.* (2022) [5], the chemical profiles and potential applications of different species within the genus *Pennisetum* exhibit significant variability. Suresh (2025) also highlighted the scarcity of GC-MS analysis in *P. polystachion*, emphasizing the need for more comprehensive research on the medicinal potential plant.



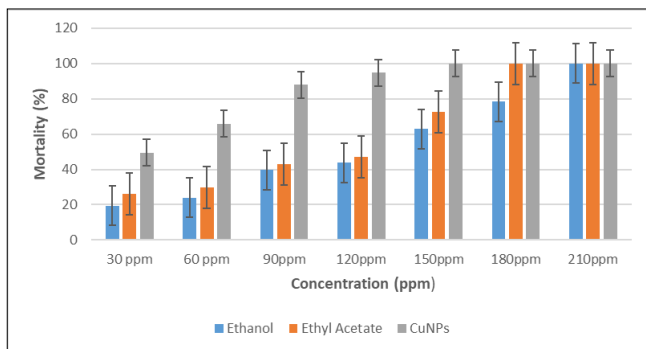
**Fig 1:** GC-MS analysis of ethanol extract of *Pennisetum setaceum*

### Mosquito (*Culex quinquefasciatus*) larvicidal activity

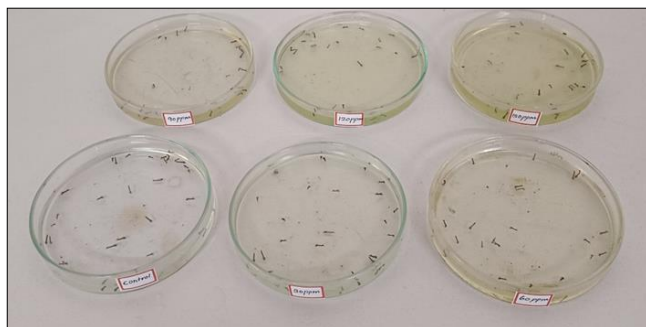
The results demonstrated a clear correlation between increased concentration and exposure time with enhanced mosquito mortality rates. At a 24-hour exposure, the ethyl acetate extract showed a peak mortality rate of 52.87% ± 0.64 at the highest concentration of 150 ppm. Ethanol extract exhibited a mortality rate of 58.84% ± 0.64 at the same concentration and time. CuNPs were particularly effective, achieving 100% mortality at concentrations of 100 ppm and above after 24 and 48 hours; the larvicidal activity was even more distinct. The ethyl acetate extract reached a mortality rate of 58.87% ± 1.56 at 150 ppm, while the ethanol extract showed a rate of 64.90% ± 1.73 at 120 ppm. The CuNPs maintained their high efficacy, causing 100% mortality at all concentrations. These findings suggest that *Pennisetum setaceum* extract, especially when formulated with CuNPs, could serve as a viable and natural alternative for mosquito control, potentially reducing the reliance on synthetic insecticides. The larvicidal activities of different substances against mosquito larvae, our study on *P. setaceum* extract showed significant mortalities at 150 ppm for ethyl acetate and ethanol extracts, respectively. This is comparable to the biosynthesized AgNPs reported by Babu *et al.* (2020) [3], which had an LC<sub>50</sub> of 32.373 ppm against *C. quinquefasciatus* larvae after 24 hours. However, CuNPs from our study demonstrated superior larvicidal potential of 100% mortality at 100 ppm. Govindarajan and Angelina (2010) reported that the methanolic fraction of *Mentha piperita* leaves exhibited an LC<sub>50</sub> value of 43.65 ppm against *C. quinquefasciatus* larvae. This indicated that *Mentha piperita* had significant larvicidal activity. Additionally, *Phyllanthus niruri* and *Letiota aspera* showed LC<sub>50</sub> values of 1819.70 and 2818.38 ppm respectively, suggesting varying levels of efficacy. Our findings suggest that *P. setaceum* extract, particularly with CuNPs, offers a potent natural mosquito control alternative, with efficacy comparable or superior to synthetic substances like AgNPs and novaluron, and highlights the potential for reducing reliance on chemical insecticides.

**Table 2:** Larvicidal activity of *Pennisetum setaceum* extract at different concentrations against *Culex quinquefasciatus*

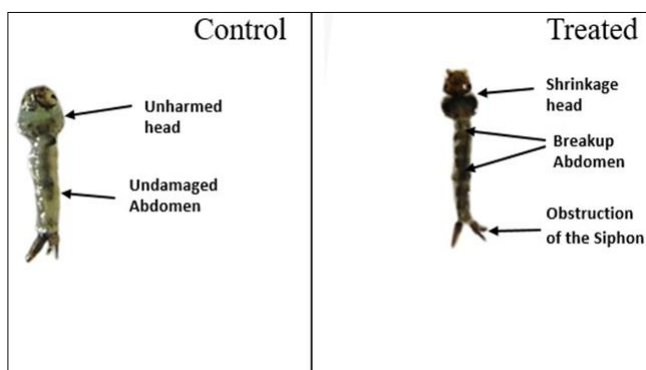
| Extract       | Exposure (Hrs) | Mortality (%) ± SD |            |            |            |            |            |
|---------------|----------------|--------------------|------------|------------|------------|------------|------------|
|               |                | Control            | 30 ppm     | 60 ppm     | 90ppm      | 120ppm     | 150ppm     |
| Ethyl Acetate | 24             | 0±0.0              | 10.03±1.24 | 15.26±0.89 | 29.38±1.33 | 37.87±1.90 | 52.87±0.64 |
|               | 48             | 0±0.0              | 12.21±1.56 | 21.23±1.34 | 32.09±2.87 | 42.78±2.45 | 58.87±1.56 |
|               | 72             | 0±0.0              | 19.33±0.65 | 23.89±2.34 | 39.66±0.23 | 43.67±0.56 | 62.83±0.72 |
| Ethanol       | 24             | 0±0.0              | 13.29±1.08 | 21.08±1.97 | 37.90±2.11 | 41.87±1.54 | 58.84±0.64 |
|               | 48             | 0±0.0              | 19.28±2.78 | 23.89±2.73 | 39.84±2.78 | 44.65±0.66 | 64.90±1.73 |
|               | 72             | 0±0.0              | 25.98±0.69 | 29.87±0.93 | 42.88±1.56 | 46.98±1.45 | 72.63±2.81 |
| CuNPs         | 24             | 0±0.0              | 33.92±1.98 | 58.56±0.06 | 74.78±1.65 | 85.76±0.76 | 100±0.00   |
|               | 48             | 0±0.0              | 46.87±0.25 | 61.62±1.92 | 84.89±0.67 | 92.76±2.87 | 100±0.00   |
|               | 72             | 0±0.0              | 49.45±2.91 | 65.90±3.45 | 87.98±2.67 | 94.75±1.89 | 100±0.00   |



**Fig 2:** Dose-dependent larvicidal activity of *Pennisetum setaceum* extract against *Culex quinquefasciatus* larvae



**Fig 3:** Larvicidal activity of *Pennisetum setaceum* extract against *Culex quinquefasciatus* larvae



**Fig 4:** Morphological damage analysis of *Culex quinquefasciatus* mosquito larvae after the treatment using CuNPs of *Pennisetum setaceum*

**Egg hatchability of *Culex quinquefasciatus***

At the lowest concentration of 1 ppm, hatchability of *C. quinquefasciatus* mosquitoes was high, ranging from 73.83% to 98.65%. However, at 1000 ppm, hatchability dropped to as low as 0% for CuNPs and as high as 15.72% for ethyl acetate in all treatments. The data indicate that

higher concentrations of the extract, particularly CuNPs, are highly effective in reducing mosquito hatchability and preventing larval formation. These findings suggest that *Pennisetum setaceum* extract could serve as a potent natural mosquito control agent, potentially reducing mosquito populations and the transmission of mosquito-borne diseases.

Comparing the ovicidal and larvicidal activities of different botanical extracts, our study on *Pennisetum setaceum* extract demonstrated a significant decreased hatchability of *Culex quinquefasciatus* mosquitoes with increasing concentrations, particularly with CuNPs, where hatchability dropped to 0% at 1000 ppm. This is in line with the findings of Balaraju *et al.* (2009) [4], who reported that the ethyl acetate extract of *Solanum chiratum* reduced egg hatchability of *C. quinquefasciatus* and *Aedes aegypti* to 12.6-25.4% and 11.4-23.4% respectively at 1000 ppm. Ramkumar *et al.* (2019) [25] also found that *C. quinquefasciatus* eggs were most susceptible to the acetone plant extract of *Calotropis baccifera*. These studies collectively suggest that botanical extracts can effectively target mosquito populations at the egg and larval stages, offering a natural alternative to synthetic insecticides. The ovicidal and larvicidal efficacies of these extracts are comparable or superior to some insect growth regulators, highlighting their potential for developing environmentally friendly mosquito control strategies.

**Table 3:** Egg hatchability of *Pennisetum setaceum* extract against *Culex quinquefasciatus*

| Concentration | Hatchability (%) |               |       |
|---------------|------------------|---------------|-------|
|               | Ethanol          | Ethyl Acetate | CuNPs |
| 1 ppm         | 98.65            | 96.74         | 73.83 |
| 10 ppm        | 95.86            | 89.67         | 67.90 |
| 100 ppm       | 70.56            | 80.74         | 59.09 |
| 200 ppm       | 50.36            | 60.88         | 50.64 |
| 300 ppm       | 40.80            | 50.78         | 47.00 |
| 500 ppm       | 35.14            | 40.93         | 32.25 |
| 700 ppm       | 25.00            | 30.82         | 9.00  |
| 900 ppm       | 15.21            | 20.82         | 0     |
| 1000 ppm      | 10.93            | 15.72         | 0     |

**Antimicrobial Activity, MIC and MBC of *Pennisetum setaceum***

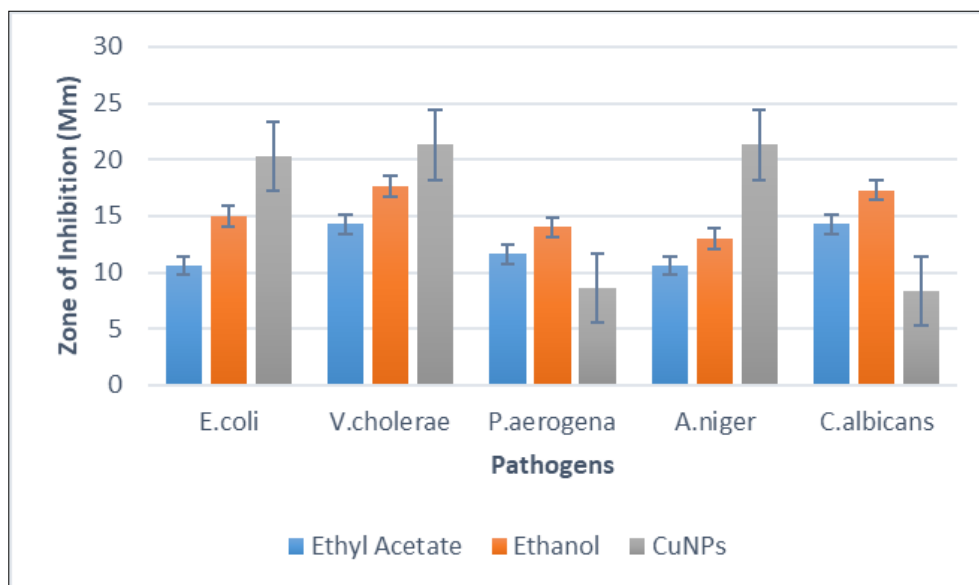
Ethyl acetate extract produced inhibition zones from 10.6±0.5 mm to 14.3±1 mm, with *V. cholerae* and *C. albicans* showing the highest susceptibility. Ethanol extract showed enhanced activity, with zones between 13±1 mm and 17.6±0.5 mm, and *V. cholerae* exhibiting maximum susceptibility. CuNPs demonstrated the most significant activity, producing zones up to 21.3±1.5 mm against *V.*

*cholerae* and *A. niger*. Compared to Modi *et al.*, 2025 [20], this study showed similar inhibitory effects against *E. coli* but expands the analysis to include additional pathogens like *V. cholerae*, *P. aeruginosa*, *A. niger*, and *C. albicans*. Unlike Jack (2020) [16], which reported zones of 6.25 mm to 13.0 mm for *P. purpureum* extracts, this study explores a wider range of pathogens and solvents, including CuNPs, suggesting a more comprehensive understanding of *Pennisetum setaceum*'s antimicrobial potential and its applicability in combating antibiotic resistance. The MIC values revealed varied inhibitory potentials among test pathogens. Ethyl acetate extract showed MIC values of 55.8-66.7%, with lowest concentration effective against *A. niger*. Ethanol extract demonstrated higher MIC values (71.8-81%), indicating greater inhibitory potential, with *V. cholerae* showing highest susceptibility at 81%. CuNPs exhibited the most promising activity with MIC values of 69.2-82.9%, peaking against *V. cholerae*, confirming enhanced antimicrobial properties versus organic extracts. Budiyananto *et al.* (2024) [7] found ethanol extracts of *Pennisetum purpureum* have potent antibacterial effects due

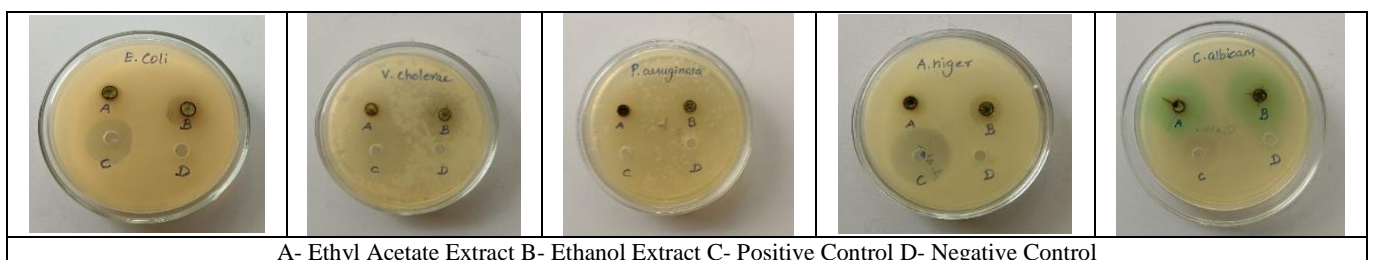
to polar phytochemicals, with lowest MIC. Chuah and Sudau (2022) [8] reported *P. purpureum* methanol extract showed strong antifungal activity against *C. gloeosporioides*. The MBC analysis of *P. setaceum* extracts and CuNPs revealed varying antimicrobial efficacy. Ethanol extract showed superior bactericidal properties with MBC values from 187.5 µg/ml to 750 µg/ml, with *E. coli* being particularly susceptible at 375 µg/ml. CuNPs exhibited MBC value of 187.5 µg/ml in *E. coli*, being most susceptible. Ethyl acetate extract had higher MBC values of 1500 µg/ml for most pathogens, except for *A. niger* at 750 µg/ml. The ethanol extract and CuNPs demonstrated more potent bactericidal properties than the ethyl acetate extract. Sapunyo *et al.* (2023) [26] reported MBC values >250 µg/mL for Abyssinica extracts against *E. coli*, *N. gonorrhoeae*, and *S. aureus*, indicating limited activity. In contrast, CuNPs in this study had an MBC of 375 µg/mL against all tested pathogens, showing superior effects. Simic *et al.* (2008) [29] found MFC values for bifonazol against fungi to be <15.0 µL/mL, suggesting strong fungicidal activity.

**Table 4:** Antimicrobial Activity, MIC, and MBC of *Pennisetum setaceum*

| Pathogens            | Ethyl Acetate |         |             | Ethanol  |         |             | CuNPs    |         |             | Positive Control (mm) |
|----------------------|---------------|---------|-------------|----------|---------|-------------|----------|---------|-------------|-----------------------|
|                      | ZOI (mm)      | MIC (%) | MBC (µg/ml) | ZOI (mm) | MIC (%) | MBC (µg/ml) | ZOI (mm) | MIC (%) | MBC (µg/ml) |                       |
| <i>E. coli</i>       | 10.6±1        | 66.7    | 1500        | 15±1     | 79.2    | 375         | 20.3±1.5 | 78.4    | 187.5       | 28±1                  |
| <i>V. cholerae</i>   | 14.3±1        | 65.2    | 1500        | 17.6±0.5 | 81      | 187.5       | 21.3±1.1 | 82.9    | 375         | 25.3±0.5              |
| <i>P. aeruginosa</i> | 11.6±0.5      | 62.0    | 1500        | 14±1.0   | 75.6    | 375         | 8.6±0.5  | 72.6    | 375         | 25.3±1.5              |
| <i>A. niger</i>      | 10.6±0.5      | 55.8    | 750         | 13±1.0   | 71.8    | 750         | 21.3±1.5 | 80.9    | 750         | 29.6±0.5              |
| <i>C. albicans</i>   | 14.3±0.5      | 57.4    | 1500        | 17.3±0.5 | 74.2    | 187.5       | 8.3±0.5  | 69.2    | 1500        | 26.6±0.5              |

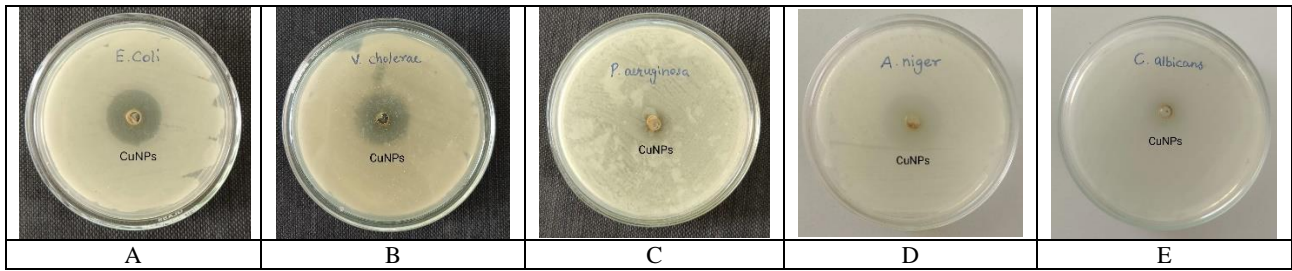


**Fig 5:** Antimicrobial Activity of *Pennisetum setaceum*



A- Ethyl Acetate Extract B- Ethanol Extract C- Positive Control D- Negative Control

**Fig 6:** Antimicrobial activity of *Pennisetum setaceum* extracts



A- *Escherichia coli* B- *Vibrio cholerae* C- *Pseudomonas aeruginosa* D- *Aspergillus niger* E- *Candida albicans*

**Fig 7:** Antimicrobial activity of CuNPs



**Fig 8:** Determination of MIC values for the *Pennisetum setaceum* against pathogenic bacteria EA- Ethyl acetate, E-ethanol, Cu NPs- Copper nanoparticles

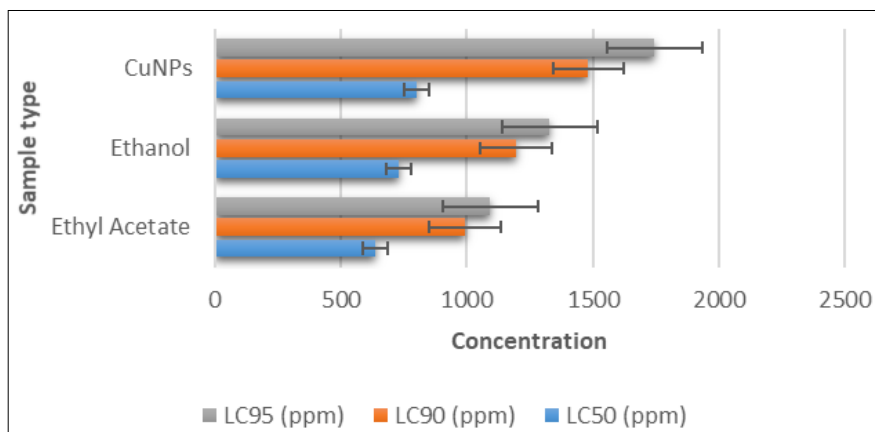
**Toxicity study using *Artemia salina***

The toxicity of *Pennisetum setaceum* extracts was evaluated using *Artemia salina*, revealing that the ethyl acetate extract had LC<sub>50</sub>, LC<sub>90</sub>, and LC<sub>95</sub> values of 634.809 ppm, 991.857 ppm, and 1093.075 ppm, respectively, while the ethanol extract exhibited higher LC values of 730.813 ppm, 1197.090 ppm, and 1329.272 ppm. These results indicate that *P. setaceum* extracts are toxic to *A. salina*. Compared to Nguta *et al.* (2012) [21], where 51% of crude extracts showed

strong cytotoxic activity at or below 100 µg/ml, *P. setaceum* extracts demonstrated lower toxicity. This is consistent with findings from Hamidi *et al.* (2014) [14], where *Aloe vera* (LC<sub>50</sub> = 3.59 µg/mL) and *Artemisia absinthium* (LC<sub>50</sub> = 15.74 µg/mL) exhibited significantly lower LC<sub>50</sub> values. Thus, *P. setaceum* extracts have higher LC<sub>50</sub> values, indicating lower toxicity relative to many plant extracts and standard toxicants.

**Table 5:** Lethal concentration of of *Pennisetum setaceum* extracts and CuNPs

| Samples              | LC <sub>50</sub> (ppm) | LC <sub>90</sub> (ppm) | LC <sub>95</sub> (ppm) |
|----------------------|------------------------|------------------------|------------------------|
| Ethyl Acetate        | 634.80                 | 991.85                 | 1093.07                |
| Ethanol              | 730.81                 | 1197.09                | 1329.27                |
| CuNPs                | 801.23                 | 1482.60                | 1744.90                |
| Potassium dichromate | 18.49                  | 25.62                  | 27.73                  |



**Fig 9:** Comparison of LC50 Values across sample typ

## Conclusion

The study demonstrated that *Pennisetum setaceum* extracts, particularly ethanol extracts, possess strong antimicrobial activity. Additionally, copper nanoparticles (CuNPs) synthesized using these extracts exhibited potent larvicidal effects against *Culex quinquefasciatus*. Phytochemical analysis of the extracts revealed the presence of compounds like tannins and flavonoids, which are known for their pesticidal properties. These findings suggest that *P. setaceum*, especially in the form of CuNPs, could serve as a natural and safer alternative for mosquito control. Furthermore, the results highlight the potential of *P. setaceum* and CuNPs as valuable resources for developing new therapeutic strategies and larvicidal bioassays.

## Conflict of Interest

The authors have no conflicts of interest to declare.

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