Uttar Pradesh Journal of Zoology

Volume 45, Issue 19, Page 90-97, 2024; Article no.UPJOZ.4160
ISSN: 0256-971X (P)

Bioefficacy of Methanolic Leaf Extracts from *Coleus amboinicus* and *Mentha spicata* against *Aedes aegypti* Mosquitoes

Srikanth Bandi [a*], Madhavi Maddala [a++], Dobbala Pavithra [b#], Mahesh Lingakari [c†] and K. Surender Reddy [d†]

[a] Department of Zoology, Osmania University, Hyderabad, Telangana State, India.
[b] Department of Zoology, Tara Govt. College (A), Sangareddy, Telangana State, India.
[c] Department of Zoology, Vivekananda Govt. Degree College (A), Vidyanagar, Hyderabad, Telangana State, India.
[d] Department of Zoology, GDC Sircilla, Rajanna Sircilla Dist., Telangana State, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i194502

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4160

Original Research Article

Received: 23/07/2024
Accepted: 27/09/2024
Published: 01/10/2024

ABSTRACT

Mosquito-borne diseases (MBDs) pose significant public health challenges globally, with India and Sub-Saharan Africa accounting for the majority of cases. *Aedes aegypti*, a primary vector of dengue fever, thrives in an environment with inadequate water supply and poor waste management. The

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[++] *Professor;*
[#] *Post-Graduate Researcher;*
[†] *Assistant Professor;*
**Corresponding author: Email: srikanthgodavari26@gmail.com;*

***Cite as:** Bandi, Srikanth, Madhavi Maddala, Dobbala Pavithra, Mahesh Lingakari, and K. Surender Reddy. 2024. "Bioefficacy of Methanolic Leaf Extracts from Coleus Amboinicus and Mentha Spicata Against Aedes Aegypti Mosquitoes". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (19):90-97. https://doi.org/10.56557/upjoz/2024/v45i194502.*

increasing resistance of mosquito population to chemical insecticides necessitates the exploration of alternative control strategies, such as botanical extracts. This study evaluates the adulticidal efficacy of methanolic leaf extracts of *Coleus amboinicus* and *Mentha spicata* against the adults of *Ae. aegypti*. Fresh leaves of both plants were collected, dried, and extracted with methanol. Phytochemical screening revealed a moderate presence of alkaloids, flavonoids, saponins, terpenoids, and glycosides in *C. amboinicus*, while phenols are highly prevalent, indicating significant biological activity. In contrast, *M. spicata* displayed a high concentration of alkaloids, flavonoids, and terpenoids, though saponins, phenols, and glycosides were less abundant. Adulticidal bioassays demonstrated that *C. amboinicus* achieved higher mortality rates across all concentrations compared to *M. spicata*. The LC_{50} value for *C. amboinicus* was 202.00 ppm, while *M. spicata* exhibited an LC_{50} of 230.00 ppm. Similarly, the LC_{90} values were 428.51 ppm and 457.40 ppm for *C. amboinicus* and *M. spicata*, respectively. These findings suggest that *C. amboinicus* has a more balanced and diverse phytochemical profile, which likely contributes to its superior adulticidal activity against *Ae. aegypti*. This study highlights the potential of *C. amboinicus* as a natural alternative for mosquito control, warranting further exploration and development for effective vector management.

Keywords: Mosquito-borne diseases; Coleus amboinicus; Mentha spicata; adulticidal activity; Aedes aegypti; phytochemical profile.

1. INTRODUCTION

Mosquito-borne diseases (MBDs) affect approximately 700 million people worldwide, causing more than one million fatalities [1]. India and sub-Saharan Africa account for nearly 80% of cases globally, with a WHO assessment indicating that 1.25 billion individuals in India are at risk [2]. MBDs are spread by the bites of infected female mosquitos. Malaria, Chikungunya, Zika, Dengue, West Nile, yellow fever, Rift Valley fever, lymphatic filariasis, and tick-borne encephalitis are among the most common illnesses transmitted by mosquitos [3]. Mosquitoes from the genera *Aedes, Culex*, and *Anopheles*, which are found in Africa, Asia, South America, and Europe, are important vectors of the pathogens that cause these illnesses [4]. These mosquitoes are the most troublesome vectors of major infections, producing illnesses including malaria, Dengue, yellow fever, filariasis, Japanese encephalitis, and Zika [5,6].

The rise of vector-borne diseases, notably dengue, is becoming a significant global health issue, exacerbated by urban expansion and poor planning. In the northeastern part of India, known for its scenic beauty and abundant mosquito vectors, dengue has emerged as a major concern, previously confined to isolated outbreaks [7]. Dengue fever is exacerbated by inadequate water supply and poor waste management, which facilitate the proliferation of the primary vector, *Aedes aegypti*. The absence of effective antiviral treatments and vaccines has led to numerous cases of dengue shock syndrome and dengue hemorrhagic fever, posing a significant public health challenge [8]. Dengue has emerged as a significant global public health issue, exacerbated by inadequate surveillance and the presence of four DENV serotypes. Dengue fever, transmitted by *Aedes* mosquitoes, is a major public health challenge in India, with an estimated 100 million new cases globally each year [9]. Tamil Nadu, Kerala, Gujarat, West Bengal, Maharashtra, and Karnataka are the most affected states, with the southern region experiencing the highest incidence, particularly in 2015 and 2013 [10].

The rising pesticide resistance in mosquito vectors impedes the development of novel treatments and vaccines. The persistent reemergence of arboviruses is caused by the failure of vector control efforts, the growth of invasive mosquitoes, and increasing human-vector interaction [11]. There are various methods in practice for controlling MBDs, essential for reducing their burden. Chemical control strategies include long-lasting insecticide-treated nets (LLINs), which are effective at preventing malaria and require no retreatment, thus reducing environmental insecticide use [12]. Indoor residual spraying (IRS) targets indoor resting mosquitoes, primarily *Ae. aegypti*, and has proven effective in reducing malaria transmission, though it requires specialized training and does not prevent bites [13], while alternatives to pyrethroids are being explored to combat resistance [14]. Peridomestic space spraying quickly reduces adult mosquito populations but is limited in controlling immature stages, necessitating continuous treatments for effective outbreak management [15]. Mosquito repellents deter mosquitoes from biting without killing them, with natural alternatives preferred to minimize environmental impacts and resistance issues [16]. However, increased pesticide use has led to resistance in mosquito populations, complicating control efforts [17].

Pesticides adversely affect pollinators like bees, contributing to a 30% decline in honey bee populations in the US and Europe and a global risk of extinction for 40% of invertebrate pollinators. This contamination extends to humans, causing serious health issues such as carcinogenicity, endocrine disruption, and neurological disorders. Studies link pesticide exposure to a 25-30% increase in cancer risk and elevated rates of leukemia and lymphoma in children. To mitigate these harmful effects, alternative approaches like integrated pest management are essential for protecting the environment and human health [18]. Concerns about non-target toxicity, residual effects, and biodegradability of these chemicals have increased interest in sustainable, eco-friendly alternatives. Botanical pesticides offer a promising solution as they are effective, readily available, inexpensive, biodegradable, and generally exhibit low toxicity to beneficial organisms [19].

The larvicidal effects of leaves and stems extracts of *Parthenium hysterophorus* on second and third instar larvae of *Ae. aegypti*. The results revealed that all extracts were effective, with the fresh leaf extract being the most potent, causing 100% larval mortality within 30 minutes. This suggests that *P. hysterophorus* leaves could serve as an effective larvicide against *Ae. aegypti* [20]. Acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of Ocimum sanctum were the most effective in causing larval mortality against *Ae. aegypti*. The lethal concentration (LC50) values were 425.94, 150.40, 350.78, 575.26, and 175.67 ppm [21]. The leaf extracts of *Argemone mexicana* were effective against the larvae of *Ae. aegypti*. At a 10% concentration, leaf extracts caused 69.57% mortality [22]. Thyme, Camphor, and Eucalyptus EOs exhibited better larvicidal activity against the 4th instar larvae of *Ae. aegypti*, but Thyme EO demonstrated superior effectiveness compared to Camphor and Eucalyptus EOs [9].

Spearmint (*Mentha spicata*), from the Lamiaceae family, is globally cultivated for its distinctive aroma and commercial value. Its essential oil, rich in compounds like carvone, carveol, dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate, is widely utilized in the flavors and fragrances industry. Beyond traditional uses, these aromatic molecules are gaining attention for their antimicrobial, antioxidant, insecticidal, antitumor, anti-inflammatory, and antidiabetic properties [23]. *Coleus aromaticus* Benth. (synonym: *C. amboinicus* Lour.; *Plectranthus aromaticus* Roxb.) is a dicotyledonous plant from the Lamiaceae family. Widely distributed across India and often cultivated in gardens, this plant holds significant folkloric medicinal value, being used by locals for treating many diseases. Studies have explored its antifungal activity [24]. *Coleus amboinicus* leaf oil was shown to be insecticidal against white termites (*Odontotermes obesus* Rhamb.), with 100% death at a dosage of 2.5×10^{-2} mg/cm3 for 5 h exposure. This was more effective than synthetic insecticides Thiodan and Primoban-20 [25].

Based on the previous studies, the present study was carried out to investigate the larvicidal efficacy of the methanolic leaf extracts of *C. amboinicus* and *M. spicata* against the 4th instar larvae of *Ae. aegypti*.

2. MATERIALS AND METHODS

C. amboinicus and *M. spicata* fresh leaves were collected from Sangareddy Town in Telangana State, India. They were cleaned completely and dried in the shade for 15 days. The dried leaves were pounded into powder with a grinder and stored in a sealed bag until needed.

Leaf powders weighing 100 g were soaked in 250 ml of methanol for four days, with frequent shaking to get the extract. On the fifth day, the solutions were filtered through Whatman filter paper no. 1 and allowed to evaporate with a circulating fan for a full day. Semi-solid extracts were collected after one day and refrigerated at 4°C until used. To make 1000 ppm stock solutions, 1 g of extracts were mixed with 10 ml of methanol and 990 ml of distilled water. The stock solutions were serially diluted to create test solutions at concentrations of 100, 200, 300, and 400 ppm. The control stock solutions were prepared using the same solvents in the same quantities but without extracts.

Several tests were conducted to identify secondary metabolites present in test solutions. The Mayor's Test was used to identify alkaloids.

The experiment involved adding Mayor's reagent, which resulted in a cream-colored precipitate. The Alkaline Reagent Test was used to identify flavonoids that turned yellow when NaOH was introduced. The Salkowski Test, which uses concentrated H_2SO_4, detects the presence of terpenoids by producing a red or orange color. Saponins were identified using the Froth Test, which includes shaking vigorously and producing froth. The Keller-Killiani Test, which involves combining HCl and $FeCl_3$ to produce a red or violet color, was used to detect glycosides. The NaOH Test, which yields a yellow color when NaOH is introduced, was previously used to detect phenol.

Ae. aegypti eggs were collected from the campus of Sangareddy by placing egg traps. The eggs were hatched in the laboratory to get the larvae. They were raised on dog biscuits and yeast powder. Adult mosquitos hatched from these larvae were fed sucrose solution soaked on cotton swabs. Adults aged 2–3 days were utilized in bioassays for the evaluation of adulticidal properties of plant extracts. The adulticidal bioassay was modified from the WHO [26] technique.

The transparent plastic bottles used in the adulticidal bioassay were washed with methanol, then distilled water and dried. The test solutions of 3 ml quantity were poured into separate test bottles along the walls. The bottles were rotated to evenly distribute the solutions over the inner surfaces. After drying, the bottles were snugly secured with lids. Twenty mosquitos were discharged in each test tube. Cotton swabs soaked in sucrose solution were inserted into the bottles. The number of dead larvae was counted for one day at intervals of 1, 6, 12, and 24 hours. The experiment was performed 3 times. The percentage of mortality was computed and corrected using Abbott's [27] method.

Results were analyzed using MS Excel's probit function. The regression analysis used a 0.05 threshold of statistical significance. The LC50 and LC90 values were calculated using regression and probit analysis.

3. RESULTS AND DISCUSSION

Fig. 1 shows the adulticidal bioassay results of methanolic leaf extracts of *C. amboinicus* and *M. spicata* against *Ae. aegypti* mosquitoes, showing the mortality percentages at various test concentrations (0, 62.5, 125, 250, and 500 ppm). At the lowest concentration of 62.5 ppm, *C. amboinicus* shows a slightly higher larval mortality (16.67 ± 2.00%) compared to M. spicata (12.99 ± 0.54%). This trend continues at 125 ppm, with *C. amboinicus* exhibiting 35.90 ± 0.67% mortality, while *M. spicata* causes 31.17 ± 0.47% mortality. As the concentration increases to 250 ppm, *C. amboinicus* again demonstrates greater effectiveness, with a mortality rate of 55.13 ± 0.75%, compared to 48.10 ± 0.90% for *M. spicata*. At the highest concentration of 500 ppm, *C. amboinicus* maintains a higher mortality rate of 82.05 ± 0.52%, whereas *M. spicata* reaches 77.92 ± 0.47%.

Figs. 2 and 3 show the results of regression analysis. The LC_{50} values indicate that *C. amboinicus* is more effective at lower concentrations, with an LC_{50} concentration of 202.00 ppm compared to 230.00 ppm for *M. spicata*. Similarly, the LC_{90} values show *C. amboinicus* at 428.51 ppm, slightly lower than *M. spicata* at 457.40 ppm. The regression equations ($y = 0.1766x + 14.326$ for *C. amboinicus* and $y = 0.1759x + 9.5426$ for *M. spicata*) and high R^2 values (0.9589 and 0.9738, respectively) indicate a strong correlation between concentration and mortality for both extracts. Overall, *C. amboinicus* consistently exhibited slightly higher mortality rates at all tested concentrations compared to *M. spicata.* The results of the phytochemical analysis reveal the reasons for the slight superiority of the *C. amboinicus* extracts over that of *M. spicata.*

Table 1 provides a detailed comparison of secondary metabolites in the methanolic leaf extracts of *C. amboinicus* and *M. spicata*, highlighting their chemical profiles. *C. amboinicus* exhibits a moderate presence of alkaloids, flavonoids, saponins, terpenoids, and glycosides. Additionally, phenols are heavily present in this extract, suggesting a significant potential for biological activities. In contrast, *M. spicata* is characterized by a high concentration of alkaloids, flavonoids, and terpenoids, all of which are heavily present. However, saponins, phenols, and glycosides are only slightly present. This distribution suggests that *C. amboinicus* has a more diverse and balanced profile of secondary metabolites. The phytochemical profile of *C. amboinicus* is balanced and diverse. Phenols and other bioactive substances found in *C. amboinicus* probably contribute to the plant's superiority in adulticidal activity as compared to the extracts of *M. spicata*.

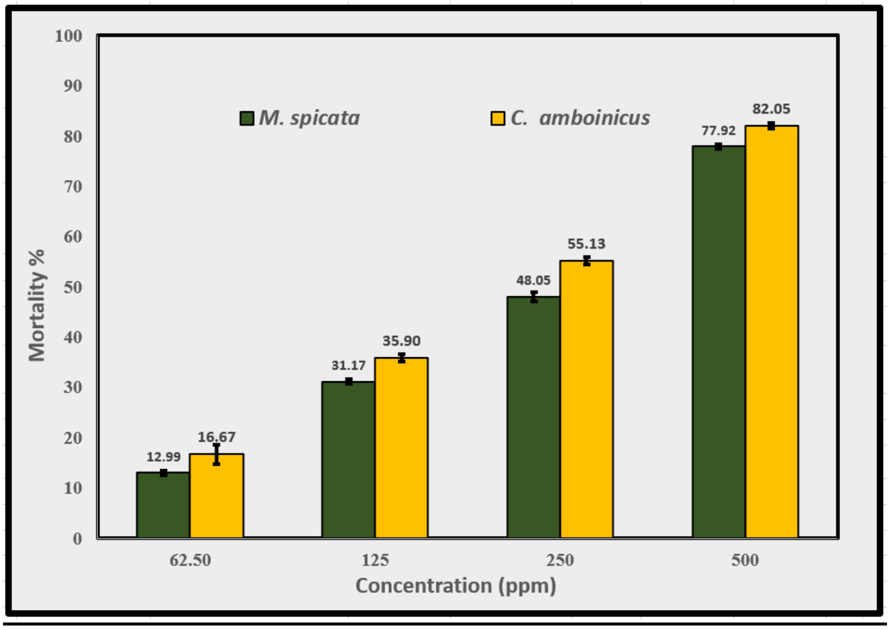

Fig. 1. Adulticidal bioassay results of methanolic leaf extracts of *C. amboinicus* & *M. spicata* against *Ae. aegypti* mosquitoes

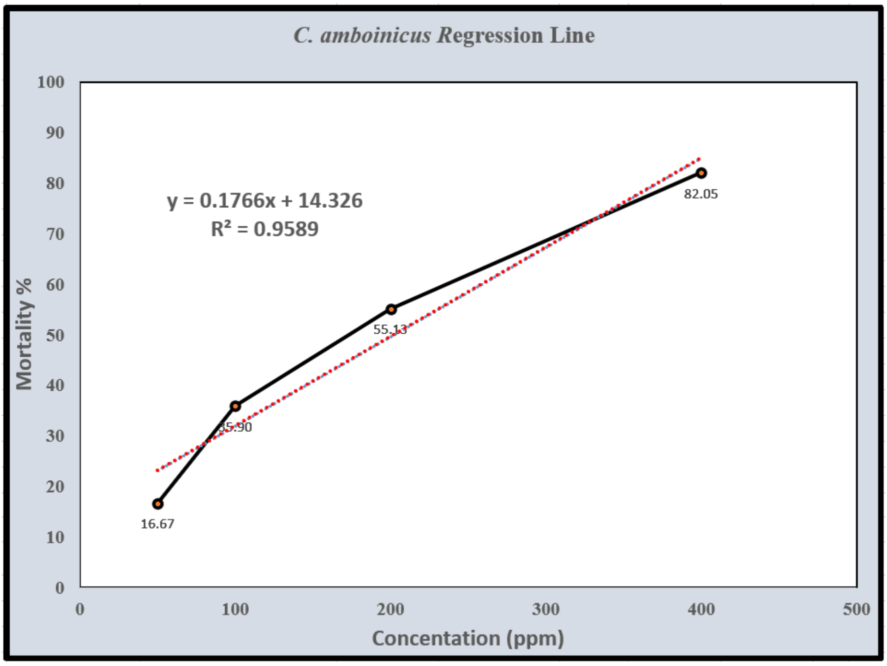

Fig. 2. Regression analysis of methanolic leaf extracts of *C. amboinicus* against *Ae. aegypti* mosquitoes

Table 1. Identified secondary metabolites in methanolic leaf extracts of *C. amboinicus* & *M. spicata*

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Phenols	Glycosides
C. amboinicus	++	++	++	++	+++	++
M. spicata	+++	+++	+	+++	+	+

Absent: -; Slightly Present: +; Moderately present: ++; Heavily present: +++

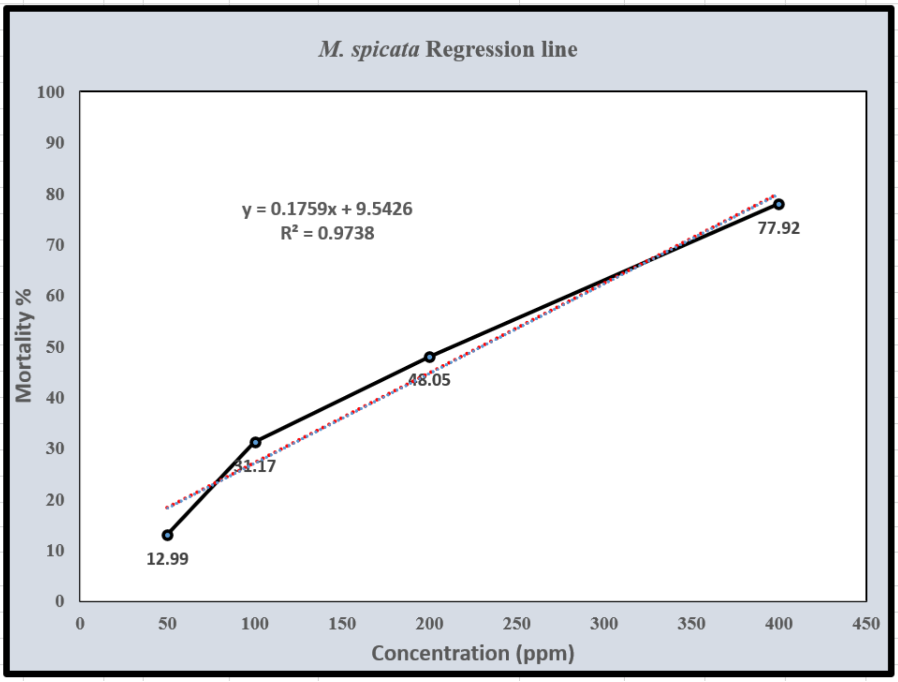

Fig. 3. Regression analysis of methanolic leaf extracts of *M. spicata* against *Ae. aegypti* mosquitoes

These findings concur with previous research that has demonstrated the antibacterial and insecticidal capabilities of *C. amboinicus* and *M. spicata*. Govindarajan et al. [28] identified 18 compounds in the essential oils of *M. spicata*, with carvone (48.60%), cis-carveol (21.30%), and limonene (11.30%) as major constituents. These oils showed significant toxicity with LC50 values of 62.62, 56.08, and 49.71 ppm, and LC90 values of 118.70, 110.28, and 100.99 ppm against the larvae of Culex quinquefasciatus, Aedes aegypti, and Anopheles stephensi, respectively. Ślusarczyk et al. [29] reported the presence of phenolic acids, flavonoids, and diterpenes along with fatty acids, including linolenic acid and docosapentaenoic acid in the extracts of *C. amboinicus.* Specific diterpenes such as acetoxydihydroxyroyleanone, dihydroxyroyleanone, rosmanol, and rosmadial presence were confirmed.

4. CONCLUSION

C. amboinicus and *M. spicata* both exhibit significant adulticidal activity against *Ae. aegypti* mosquitoes, but it seems that *C. amboinicus* is more effective, probably because of the variety of secondary metabolites it contains. The findings of this investigation imply that *C. amboinicus* should be investigated further as a potent natural larvicide for mosquito control. Future research ought to look into the processes underlying these extracts' actions as well as any potential synergistic effects they might have when paired with other botanical insecticides.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.3390/molecules26102915

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4160