

Eco-Guard: Innovative Herbal Antidotes for Mosquito Control

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ABSTRACT

Mosquitoes pose a significant threat to human health as vectors of diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, and yellow fever. Conventional mosquito control methods, including chemical liquid vaporizers and synthetic insecticides, can cause ecological imbalances, environmental pollution, and harm to nontarget organisms. Additionally, these methods can be prohibitively expensive for those living below the poverty line. Therefore, safer and more biodegradable alternatives are necessary. This research explores the use of plant extracts as a natural and effective mosquito repellent. Citrus peel extract, containing bioactive chemicals such as alkaloids, flavonoids, tannins, and phenolic compounds, disrupts the host-seeking ability of mosquitoes by impacting their nervous system. The scent of citrus affects mosquito behaviour and leads to neural collapse upon contact. Artemisia pallens, known for its antimicrobial properties and pleasant fragrance, is also employed. Moreover, A. calamus rhizome exhibits a variety of pharmacological responses, including sedative, CNS depressant, anticonvulsant, antispasmodic, cardiovascular, hypolipidemic, immunosuppressive, anti-inflammatory, cryoprotective, antioxidant, antidiarrheal, antimicrobial, anticancer, and antidiabetic activities. The study discusses the ethanol extraction process of these plant materials to develop an effective mosquito repellent. By utilizing these natural ingredients, we aim to provide an affordable, nonhazardous, and environmentally friendly alternative to synthetic insecticides (4). This herbal mosquito repellent not only offers immediate relief from mosquito bites but also holds potential for broader applications as a general insecticide. The findings underscore the importance of integrating traditional botanical knowledge with modern scientific approaches to address global health challenges posed by mosquitoes. Further research is warranted to optimize the formulation and expand its use against other insect pests.

INTRODUCTION

Mosquitoes breed in various types of standing water, which provide an ideal environment for laying eggs and developing into larvae. Common breeding sites include puddles, clogged gutters, open containers, ditches, drains, natural water bodies, and rain barrels. Small puddles of water, such as those formed after rainfall, can serve as breeding grounds for mosquitoes, making it essential to empty or cover containers to prevent water accumulation (1). Areas with poor drainage or blocked ditches are particularly susceptible to water accumulation, creating suitable environments for mosquito breeding. Mosquitoes typically prefer still or stagnant water overflowing water, necessitating the regular elimination or treatment of standing water sources, proper drainage maintenance, and the use of mosquito control methods like larvicides or biological controls (3). Mosquitoes are vectors for a variety of dangerous diseases, posing significant health risks globally. Malaria, caused by Plasmodium parasites and transmitted by Anopheles mosquitoes, remains a leading cause of illness and mortality in many tropical regions. Dengue fever, spread by Aedes mosquitoes, can lead to severe flu-like symptoms and, in extreme cases, hemorrhagic fever (5). The Zika virus, also transmitted by Aedes mosquitoes, can cause birth defects and neurological complications. Additional mosquito-borne illnesses include yellow fever, West Nile virus, chikungunya, and Japanese encephalitis, each with unique symptoms and potential complications (2). Preventive measures such as mosquito control, the use of bed nets, and personal protection are crucial in mitigating the spread of these diseases and safeguarding public health. Different mosquito species are responsible for various mosquito-borne diseases. For instance, chikungunya virus is spread by Aedes *aegypti* and *Aedes albopictus*; dengue virus by *Aedes aegypti* and *Aedes albopictus*; Eastern equine encephalitis virus by *Culiseta melanura*; malaria by female Anopheles mosquitoes; yellow fever by Aedes or Haemagogus species; and Zika virus by Aedes species (6).

Phytochemical extracts from plants like Citrus limetta contain active ingredients such as alkaloids, flavonoids, saponins, phenolics, and tannins. These compounds may inhibit lactic acid receptor cells, potentially altering or masking the lactic acids that attract mosquitoes, thus interfering with the mosquitoes' ability to detect the host's scent (9). Artemisia pallens, known for its potent anti-malarial properties, contains terpenoids and flavonoids responsible for its toxicity against mosquitoes. Similarly, Acorus calamus, commonly known as sweet flag, has a traditional reputation for repelling insects due to its aromatic nature, with its essential oils containing flavonoids and phenolic compounds believed to have insect-repelling properties (7). Traditional methods for mosquito control have included the combustion of dried leaves from Azadirachta indica, which produces smoke used since ancient times to combat mosquitoes. Ethnobotanists report that villagers apply plant materials to their skin as repellents and use smoke to keep mosquitoes out of homes. In rural areas, the practice of burning various plant parts, especially leaves, remains common. Other methods include suspending plants within households and dispersing leaves across floors. In China, rural populations burn herbs like Artemisia and calamus to repel mosquitoes and protect livestock. Traditional insect or mosquito repellent plants are widely known and utilized globally. Chemical mosquito repellents, developed post-World War II, primarily rely on DEET, picaridin, and permethrin. While effective, these chemicals pose health risks, including respiratory issues, skin allergies, and potential neurological effects from compounds like prallethrin and transfluthrin. Consequently, there is a pressing need to develop plant-based mosquito repellents that are both effective and safe, promoting their use among local communities for enhanced human and environmental health. The focus of this study is to pioneer the development of plant-based mosquito repellent solutions that are both effective in repelling mosquitoes and safe for human use, highlighting the need for and benefits of bio-products in providing effective control against mosquito bites while ensuring the safety of humans and the environment.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials for Repellent Formulation

The bark of Sweet Flag was collected from a forest area at coordinates 12° 58' 34" North, 80° 11' 1" East. Citrus peel, obtained as waste from fruit juice processing, was gathered

| Extract (µl) | Zone(cm) |
|--------------|----------|
| 25 | 0.6 |
| 50 | 1.5 |
| 75 | 1.6 |
| 100 | 1.7 |

Escherichia coli

from locations in and around Chennai $(13.0827^{\circ}N, 80.2707^{\circ}E)$. Artemisia pallens was sourced from a floral shop. All plant materials were thoroughly washed with distilled water to remove sand and other impurities. They were then air-dried in the shade for 17 days at a temperature range of 25-35°C.

2.2 Preparation Of Plant Materials

The dried plant materials were pulverized into a fine powder using an electric stainless steel blender. Twenty-five grams of the powdered Sweet flag were weighed and macerated in 100 ml of ethanol for seven days. Similarly, twenty-five grams of Citrus peel and *Artemisia pallens* were also weighed and macerated in 100 ml of ethanol for seven days. The resulting crude extracts were filtered through Whatman No.1 filter paper to separate the liquid plant extracts, which were then collected in a container.

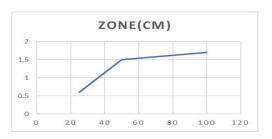
2.3 Combination of Plant Extracts

The plant extracts of Citrus peel, *Artemisia pallens*, and Sweet flag were mixed in equal proportions. The ethanol was then evaporated by placing the mixture in a hot air oven for approximately 3-4 hours.

3. RESULTS AND DISCUSSION

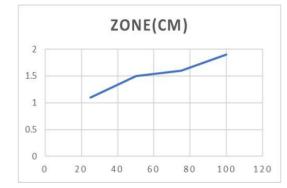
The antimicrobial efficacy of the extracts was evaluated through an antibacterial activity test using the plating method. This test involved assessing the effectiveness of the extracts at various concentrations against different bacterial strains, specifically *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. For the antibacterial activity test, each bacterial strain was cultured and evenly spread on separate agar plates. The plant extracts of Citrus peel, *Artemisia pallens*, and Sweet flag, prepared in different concentrations, were then introduced to the agar plates containing the bacterial cultures. The plates were incubated at an optimal temperature of 37°C for 24 hours to allow bacterial growth and interaction with the extracts.

The results were assessed by measuring the zones of inhibition around each extract application site. A clear zone of inhibition indicated the antimicrobial activity of the extracts against the respective bacterial strain. The diameter of these zones was measured in centimeters (cm) to quantify the level of antibacterial effectiveness. In addition to the antibacterial activity test, the mortality rate percentage of the bacterial strains was also evaluated to further understand the efficacy of the extracts. The bacterial cultures were exposed to different concentrations of the extracts, and the mortality rates were recorded after a specific period. This allowed for a comprehensive assessment of the bactericidal properties of the extracts.



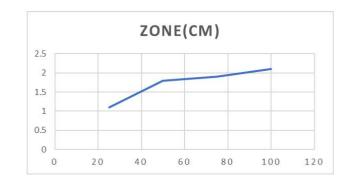
Staphylococcus aureus

| Extract (µI) | Zone(cm) |
|--------------|----------|
| 25 | 1.1 |
| 50 | 1.5 |
| 75 | 1.6 |
| 100 | 1.9 |



Pseudomonas aeruginosa

| Extract (µI) | Zone(cm) |
|--------------|----------|
| 25 | 1.1 |
| 50 | 1.8 |
| 75 | 1.9 |
| 100 | 2.1 |



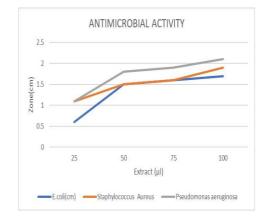
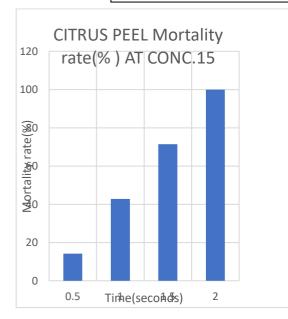
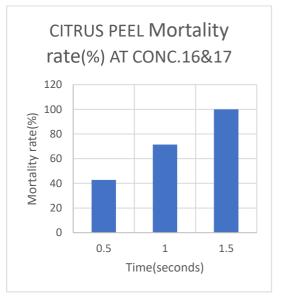


Figure 1. Antimicrobial activity of different organism

| Contents | % Mortality at different time intervals in seconds | | | | | | | | | | |
|--|--|------|------|------|------|------|------|------|------|------|-----|
| Extract | Concentrati on(%) | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 |
| Citrus peel | 15 | 14.2 | 42.8 | 71.4 | 100 | | | | | | |
| | 16 | 42.8 | 71.4 | 100 | | | | | | | |
| | 17 | 42.8 | 71.4 | 100 | | | | | | | |
| Artemisia pallens | 19 | 14.2 | 28.5 | 42.8 | 4.28 | 71.4 | 71.4 | 100 | | | |
| | 20 | 28.5 | 57.1 | 71.4 | 71.4 | 100 | 100 | 100 | | | |
| | 21 | 28.5 | 57.1 | 71.4 | 71.4 | 100 | 100 | 100 | | | |
| Sweet Flag | 16 | 0 | 0 | 14.2 | 28.5 | 42.8 | 42.8 | 57.1 | 71.4 | 71.4 | 100 |
| | 17 | 14.2 | 14.2 | 28.5 | 28.5 | 57.1 | 71.4 | 71.4 | 100 | | |
| | 18 | 14.2 | 14.2 | 28.5 | 28.5 | 57.1 | 71.4 | 100 | 100 | | |
| Mixture of 3 component s(Citrus peel 16% +Artemisia pallens 20%+sweet flag | | 42.8 | 100 | | | | | | | | |
| 20%+sweet flag 17%) | | | | | | | | | | | |

Table 2: Mortality Rate at Different Time Intervals





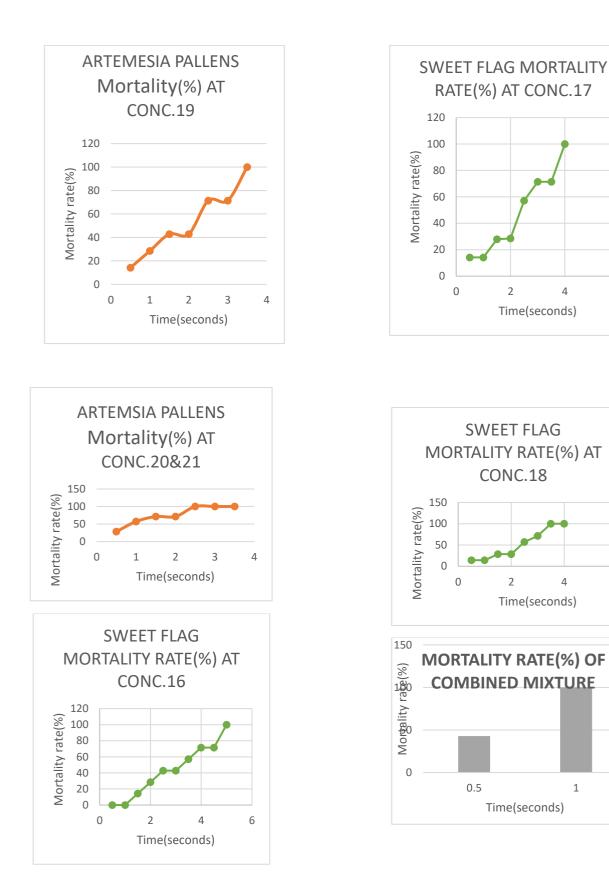


Figure 3. Mortality rate percentage of different samples at different concentration

| Repello(ml) | Time in seconds | Mortality rate(%) |
|-------------|------------------|-------------------|
| 0.5 | 4 | 85 |
| 1 | <mark>3.5</mark> | 87.6 |
| 1.5 | 3 | 89 |
| 2 | 2.4 | 92 |
| 2.5 | 2 | 95 |
| 3 | 1 | 99 |

Table 1: Repello's efficiency

CONCLUSION

In this study, the mixture of plant extracts demonstrated broadspectrum antibacterial efficacy against both gram-positive and gram-negative bacteria. This indicates that the plant extracts themselves could act as preservatives, contributing to a good shelf life. Most commercially available mosquito repellents contain non-biodegradable synthetic chemicals such as N,N-diethyl-3methylbenzamide (DEET), dimethyl phthalate (DMP), and allethrin, which pose potential environmental hazards. With increasing concerns for public safety, there is a growing interest in utilizing plant-derived natural products. This interest is driven by the effectiveness, environmental friendliness, and biodegradability of these natural products, aligning with contemporary priorities for sustainable solutions (8). Traditional methods of burning plant materials, such as Ocimum tenuiflorum, dried fibers of Cocos nucifera, and barks of Cedrus deodara, have efficiently repelled mosquitoes. However, the combustion of plant materials emits various small particles and gases, including carbon monoxide, nitrogen dioxide, formaldehyde, and carcinogenic compounds such as benzo[a]pyrene and benzene (10), which may be harmful to the environment. In this paper, we combined the extracts of Citrus limetta, Artemisia pallens, and Sweet flag, all of which contain active constituents such as flavonoids, terpenoids, and other bioactive compounds. This formulation is expected to be an effective mosquito repellent. The preparation of the extracts was conducted under sterile conditions using a straightforward method. The resulting product has a shelf life of approximately 12 months, after which it may lose its effectiveness against mosquitoes. This approach can be easily scaled up for production in rural areas, providing a viable and sustainable solution for mosquito control.

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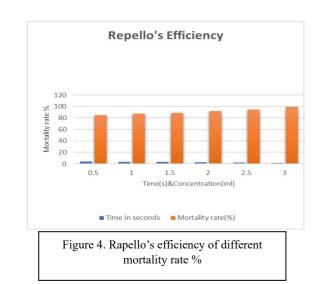
COMPETING INTERESTS:

No conflicts of interests

DATA AVAILABILITY STATEMENT:

The sequence is not reported in any of the previous studies. So, the data availability statement is not applicable.

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