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Exploring the Larvicidal Efficacy of Extracts from Red Lady Variety Carica Papaya Leaves Against Mosquito Vectors

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ABSTRACT

Background: Carica papaya L. (Red Lady variety) contains flavonoids, alkaloids, and polyphenolic compounds in its fresh leaves, which have medicinal properties and serve as safe natural larvicides.
Objective: This study aims to investigate the larvicidal potential of acetone and n-hexane extracts from red lady variety Carica papaya leaves compared with permethrin.
Materials and Methods: The Red Lady leaves extraction process was conducted at the University of Karachi Pakistan. Initially, 5kg of fresh Green was collected, cleaned, washed thoroughly with tap and distilled water before being air-dried, chopped, and blended. Acetone/n-hexane was used as solvents in a Soxhlet extractor. The samples were used in the Dengue Mosquito Vector Assay, where Aedes aegypti specimens were sourced from egg rafts. The larvae were fed fish feed pellets until they molted into third-instar larvae. The plant extract was administered in serial concentrations and, a stock solution of 200ppm was created and 10 larvae were transferred to the flask containing the test solution. The larvae were exposed to the test solution 6 times, with permethrin as the positive-control and ethanol as the negative-control.
Results: The n-hexane extract exhibited remarkable efficacy, causing a 90% mortality rate among larvae at a concentration of 200µg/ml. In contrast, the acetone extract showed lower effectiveness, resulting in only a 10% mortality rate at the same concentration. In contrast, the standard drug Permethrin achieved a 100% mortality rate.
Conclusion: The n-hexane extract is a potential larvicide against Aedes aegypti larvae, while more research is needed to optimize the efficacy of the acetone extract.
Keywords: Acetone Extracts, Carica Papaya, Larvicidal, n-Hexane Extracts, Red Lady.

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INTRODUCTION

Aedes mosquitoes spread the viral infection dengue, which has emerged as a significant global health concern, primarily in tropical regions.[1] Pakistan, being tropical and biodiversity-rich, hosts a plethora of medicinal plants. However, traditional remedies have been replaced by modern chemical drugs. The tropical climate in Pakistan not only fosters the growth of diverse plants but also serves as an optimal breeding ground for disease vectors, principally mosquitoes. Mosquitoes, classified into three sub-families—Toxorhynchitinae, Culicinae, and Anophelinae—include blood-sucking species, for instance, Aedes, Culex, Anopheles, and Mansonia, known to spread diseases like elephantiasis, malaria, and dengue fever.[2]

The dengue virus (DENV), a member of the Flaviviridae family, comprises five serotypes (DENV-1 to 5). The fifth serotype has mostly disseminated among non-human primates and mosquitoes also known as the sylvatic cycle, with only one reported human infection case in 2013.[3,4] Aedes niveus is the preferred vector for DENV-5, whereas Aedes aegypti commonly transmits other serotypes.[5] Female mosquitoes, needing external blood for egg production and development, mainly rely on iron-rich blood.[6] Although the transmission rate of DENV-5 is low and scientific knowledge about it remains limited, the World Health Organization primarily attributes dengue to the other four serotypes.[7]

Dengue infections can be primary or secondary, with severe cases most important to potentially fatal dengue hemorrhagic fever. In the face of the life-threatening nature of severe dengue, specific anti-viral medications remain unavailable.[8] Therefore, controlling mosquito vectors is crucial for disease prevention. Efforts to diminish vector populations involve biological agents (natural enemies) or chemical compounds. At present, chemical control, such as repellent lotions or liquid sprayers useful at larval breeding sites, is the standard protocol.[9] On the other hand, prolonged exposure has led to resistance, requiring the development of eco-friendly bio-larvicides derived from plant materials.[10] Plenty of medicinal plants have facilitated the search for larvicidal agents, with Carica papaya showing significant potential.[11]

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Papaya (Carica papaya L.) is a fruit-bearing plant of the Caricaceae family. It is a large perennial herb native to Central America that grows at a rapid rate. Brazil, Indonesia, Mexico, and Nigeria are among the world's leading papaya producers.[12] Papaya is extremely nutritious, high in antioxidants, and has significant medicinal properties. It contains provitamin A, carotenoids, vitamin C, vitamin B, lycopene, dietary minerals, and dietary fibers, making it a good source of nutrients. Additionally, papaya peel is used in cosmetics. Papaya latex has antimicrobial, anthelmintic, antimalarial, antifungal, anti-amoebic, hepatoprotective, anti-fertility (male and female), immune-modulatory, and anti-histaminic properties.[13]

The leaves of the papaya plant have several medicinal properties, including potent anti-cancer, anti-oxidative, anti-inflammatory, anti-microbial, and anti-sickling effects.[14] They also provide nephron-protective, 9 hepatoprotective, hypoglycemic, and hypolipidemic properties against toxins in the human body. These leaves contain active components such as carpaine, pseudoscorpion, dehydrocarpaine I and II, choline, carposide, vitamin C, and carotenoids like β-carotene, lycopene, and anthraquinones.[15]

Conventionally, the juice from tender papaya leaves has been used as a home medication for dengue fever, and now papaya leaf extracts are available in capsule form.[15] The leaves, seeds, and latex of papaya are also utilized to treat digestive issues, thanks to the protein-digesting enzyme papain, which aids natural digestion.[16] Papain and carpaine alkaloids found in papaya leaves interrupt larval growth, leading to mortality. In addition, natural products offer advantages such as bio-degradability and selectivity toward pathogenic larvae.[2]

Hence, the current study aims to explore the larvicidal potential of Red Lady Variety Carica Papaya Leaves, presenting an innovative approach to mosquito vector control.

MATERIALS AND METHODS

The in vitro experimental study took place at the Department of Pharmacology and Therapeutics, Baqai Medical College/University of Karachi, where in-vitro techniques were employed. The research lasted for duration of 6 months from 15th September 2023 to 15th March 2024

Ethical Approval

The ethical approval was taken from Baqai Medical University on 2nd August 2023(Ref: BMU-IREB/03-2023).

Plant Collection and Identification

Samples of the Carica papaya 'Red Lady' variety (CPL. var. RL) were collected from recent harvests at the University of Karachi and underwent authentication by an ethno-botanist. An official identification code (G.H. No. 97627) was assigned to the plant, which was then preserved in the Botanic Garden and Herbarium Department at the University of Karachi for documentation and reference purposes.

Plant Material Extraction

The Carica papaya 'Red Lady' variety (CPL var. RL) extraction process was carried out at the H.E.J. Research Institute of Chemistry's Industrial Analytical Centre (IAC) at the University of Karachi, Pakistan. Initially, 5 kg of fresh Green Carica papaya 'Red Lady' variety were collected, cleaned, and ready. The leaves were washed thoroughly with tap and distilled water before being air-dried, chopped, and blended. Acetone and n-hexane were used as solvents in a Soxhlet extractor, with acetone and n-hexane extractions requiring 10 and 82 grams of dried sample, respectively.[17,18] The 72-hour extraction process involved circulating the solvent vapour through the sample, followed by condensation and collection of the extract. The extracts were then concentrated under reduced pressure at 60 °C, using a Buchi Rotavapor R-200, dried, and stored in a refrigerator at around 4°C and using them within 1 to 2 weeks for further analysis.[19] This meticulous method ensured the comprehensive extraction of components, preparing them for subsequent investigation.

Dengue Mosquito Vector Assay

In the experiment, a mosquito dipper (BioQuip, USA) and stainless steel trays (35x30x5 cm) were used along-side glass beakers. For chemical testing, Dimethyl sulfoxide ReagentPlus® (≥99.5%, SIGMA-ALDRIC, D5879-2.5L, Lot # SHBR5702) and Permethrin 5% W/W (GlaxoSmithKline Pakistan Limited, M.L. No. 000017, R. No. 037852) were utilized. In additionally, the larvae were fed with Osaka Green Fish Food, which has a nutritional composition of 3% crude fat, 4% crude fiber, and 28% crude protein.

Aedes aegypti specimens were obtained from the research laboratory of the International Center for Chemical and Biological Sciences (ICCBS) at the HEJ Research Institute of Chemistry, University of Karachi, Pakistan. These specimens were acquired from egg rafts kept in glass-ware filled with distilled water two days preceding to the assay. Upon hatching into first-instar larvae, the eggs were provided with "pellet," a type of fish feed, until they molted two times into third-instar larvae. Larval identification in larvicidal assays is based on well-defined morphological features in mosquito larvae, which may include the shape of the body or the form of mouthparts visible to the under microscopic examination. Behavioral characteristics, such as swimming and feeding habits, also aid in the identification process. For the duration of the larvicidal assay, each fraction was applied in serial concentrations to the third-instar larvae. A stock solution of 200 ppm was made by mixing 100 mg of

plant extract in 5 mL of ethanol. From this stock solution, 0.5 mL was mixed with 49.5 mL of distilled water to create a test solution of 200 ppm. Afterward, batches of ten larvae were transferred to the flask containing the test solution. Each experiment was replicated six times. Permethrin (5% W/W (GlaxoSmithKline Pakistan Limited, M.L. No. 000017, R. No. 037852), Expiry Date: 31st December 2025 Registration Number: 037852) was employed as the positive control, while ethanol (Dimethyl sulfoxide ReagentPlus® (≥99.5%, SIGMA-ALDRICH, D5879-2.5L, Lot # SHBR5702), Expiry Date: 30th June 2026, Lot Number: SHBR5702. served as the negative control. Percentage mortality was determined after 24 hours using the following formula.[2]

$$\% \text{ Mortality} = \frac{\% \text{ survival in untreated control} - \% \text{ survival in treated control sample}}{\% \text{ survival in untreated control}} \times 100$$

RESULTS

The Dengue Mosquito Vector Assay is instrumental in screening and identifying promising candidates for controlling dengue vector populations. It offers valuable approaching into the efficacy of different components and formulations, assisting in the development of novel mosquito control strategies and interventions intended at alleviating the burden of dengue fever and other mosquito-borne illnesses.

The initial screening denoted the larvicidal potential of crude plant extracts, which can be attributed to their various mixtures of active compounds. Table 01 presents the varied effectiveness of different solvents used in extracting compounds from Carica papaya leaves, particularly the Red Lady variety. Two solvents were tested against third instar larvae of Aedes aegypti.

Table 01 shows the larvicidal activity of different concentrations of various CPL extracts (RL). At a concentration of 200 µg/ml, the acetone extract of CPL resulted in 10% mortality, with 1 larva dead and 9 alive. The n-hexane extract of CPL, at the same concentration, exhibited a significantly higher mortality rate of 90%, with 9 larvae dead and 1 alive. In comparison, the standard Permethrin at a concentration of 10 µg/ml achieved 100% mortality, with all 10 larvae dead (p-value = 0.031). These results suggest varying efficacy of the extracts, with the n-hexane extract showing considerable larvicidal potential.

Table 01. Larvicidal Activity of Difference Conc. of Various CPL Ext. (RL)

Sample Code	Consentration	% Mortality	Dead	Alive	P-Value
Acetone ext. of CPL. var. RL	200µg/ml	10%	1	9	0.031
N-Hexane ext. of CPL. var. RL	200µg/ml	90%	9	1	
Permethrin (Std)	10µg/ml	100%	10	0	

DISCUSSION

Mosquitoes are the primary vectors of pathogenic organisms. It is vital to comprehend the spatiotemporal distribution of mosquito-borne infection risks to develop and implement efficient control strategies.[20]

A wide variety of aquatic environments can be colonized by them, such as rice fields, lakes, wetlands, rivers and riverbanks, saltwater fields, contaminated water in sewage disposal systems, rock pools, tree cavities, abandoned household containers, and plant axils.[21]

Mosquitoes are significant vectors of a number of tropical ailments in humans, such as malaria, filariasis, and numerous viral diseases, including dengue, dengue hemorrhagic fever, yellow fever, and Japanese encephalitis. Approximately two billion people worldwide live in areas where these ailments are endemic.[22]

The discussion emphasizes the essential part of larvicidal activity within incorporated mosquito management strategies, which are considered to alleviate mosquito populations and diminish the risk of mosquito-borne ailments. Effective larvicides, when employed alongside other control measures like adulticidal spraying and source reduction, are instrumental in limitation disease transmission and safeguarding public health. This study revealed distinct outcomes concerning the efficacy of papaya leaf extract as a larvicide. At the same time as the n-hexane extract demonstrated promising activity, the acetone extract displayed negligible effectiveness. In 2020, Sudarwati TP reported an LC_{50} value of 4929.344 ppm. These results suggest that papaya leaves possess larvicidal activities against Aedes aegypti, which can aid in disrupting the development cycle of these mosquitoes.[2]

Another research in 2019, Ilham R et al Determined that the lethal concentration 50 (LC^{50}) of the ethanol extract from papaya leaves was 215.964 ppm or 0.021%.[23] The extract established efficacy as a larvicide against Aedes spp. instar III and IV, with an LT_{50} of 2369.642 minutes.[24]

Mosquito larvae in the L3-L4 instar stages were exposed to dosage of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, and 50 mg/ml of each prepared larvicide

(leaf and seed extract of Carica papaya) for 24 hours, and the percentage mean survival of the larvae was recorded. At a dosage of 10 mg/ml, 100% of the larvae were dead within 3 to 12 hours of exposure to Carica papaya's seed and leaf extracts, effectively inhibiting mosquito growth and development.[25]

Our findings highlighted that papaya leaf extracts protect lethal effects on Aedes spp. larvae, demonstrating their potential as larvicides. On the other hand, additional investigation is essential to establish the optimal dosage for accomplish larvicidal efficacy. In addition, exploring the impact of these extracts on larvae of various species is crucial, as species-specific factors may influence mortality rates in response to the extract's insecticidal properties. Biological variables such as the geographical origin of the plant, storage conditions, and plant maturity could also affect efficacy. Furthermore, environmental factors like temperature, humidity, and water pH may influence larval susceptibility to the extract.

It is crucial to comprehend these complex dynamics to improve larvicidal strategies and guarantee reliable results from vector control initiatives. More research should be done on these points to improve the use of papaya leaf extracts as larvicides in various environmental contexts and target species.

CONCLUSION

The acetone extract showed minimal impact on larvae, whereas the n-hexane extract had significant larvicidal activity. Non-polar solvents like n-hexane should be prioritized over polar ones, such as acetone, due to their enhanced effectiveness. This underscores the importance of solvent selection in influencing larvicidal properties

Recommendations

The results of the study show that the larvicidal activity of papaya leaf extracts in n-hexane and acetone differs significantly. Acetone was only moderately effective against the targeted larvae, while n-hexane demonstrated possible effects. This demonstrates how the choice of extraction solvent affects the larvicidal qualities of the resulting extract. To better understand the mechanisms underlying the varying efficacy of extracts and to support the development of more potent formulations derived from papaya leaves that are larvicidal, more research into the individual compounds in each extract and their effects on mosquito larvae is advised.

Ethical consideration

This study was approved by Institutional Review Board of Baqai Medical University (BMU-IREB/03-2023) Karachi, Pakistan.

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Conflict of Interest

Authors declared no conflict of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTION

The following authors fulfill authorship criteria as per ICMJE guidelines;

Asadullah, Ahmed A: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Bijarani AN, Zafar U: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

Imran S: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.

Sheikh SA: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.

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