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PREPARATION OF STABLE EFFERVESCENT GRANULES FROM THE EXTRACT OF CARICA PAPAYA FOR THE TREATMENT OF THROMBOCYTOPENIA ASSOCIATED WITH DENGUE

Original Research

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ABSTRACT

Background: Dengue fever is a rapidly spreading mosquito-borne viral infection that presents with severe symptoms, including thrombocytopenia and platelet dysfunction. Due to the absence of a definitive antiviral treatment or widely available vaccine, supportive therapies are critical in managing the disease. Medicinal plants such as *Carica papaya* have shown therapeutic promise in restoring platelet counts due to their bioactive compounds like papain. However, traditional dosage forms of papaya extract have limitations in stability and patient compliance.

Objective: To develop and evaluate stable effervescent granules from *Carica papaya* leaf extract with improved patient acceptability and optimized pharmaceutical properties.

Methods: Fresh leaves of *Carica papaya* were collected, dried, and powdered. Aqueous and alcoholic extracts were prepared using maceration methods. Effervescent granules were formulated using citric acid, tartaric acid, and sodium bicarbonate, incorporating various doses: 2g/15mL and 3g/20mL (alcoholic extract), 5g powdered drug, and water extract. Granules were evaluated for pH, effervescence time, particle size distribution, angle of repose, compressibility index, and moisture content. Stability testing was conducted for up to six months.

Results: The 3g/20mL alcoholic extract granules demonstrated the best performance, with effervescence onset within 1–2 seconds and pH of 5.0, falling within pharmacopeial range. These granules showed good flow properties with an angle of repose <30° and compressibility index of 13%. Moisture content was 2.7%, and particle size distribution revealed 85.3% retention on sieve no. 40. In contrast, water-based granules lost stability within 24 hours and failed to produce effervescence after storage.

Conclusion: Effervescent granules formulated with 3g/20mL alcoholic extract of *Carica papaya* exhibited optimal pharmaceutical characteristics and stability, making them a promising candidate for supportive dengue therapy.

Keywords: Carica papaya, Dengue, Effervescent granules, Papain, Pharmaceutical stability, Thrombocytopenia, Traditional medicine.

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INTRODUCTION

Medicinal plants have long played a pivotal role in global healthcare, serving as a rich source of therapeutic agents derived from their phytochemical constituents. These natural compounds are not only vital in traditional medicine but also contribute significantly to the development of novel pharmacological agents aimed at promoting health and preventing disease, including cancer. In recent years, there has been a resurgence of interest in phytotherapy, particularly in low- and middle-income countries, where medicinal plants offer a cost-effective and accessible alternative to synthetic drugs. Among the numerous public health threats, dengue fever remains one of the most critical challenges worldwide. Caused by infection with one of the four dengue virus serotypes (DEN-1 to DEN-4), the disease is transmitted predominantly by the *Aedes aegypti* mosquito and manifests with a spectrum of clinical symptoms ranging from mild fever, headache, and joint pain to severe hemorrhagic complications and plasma leakage (1,2). A key hematological feature of dengue infection is thrombocytopenia, a rapid decline in platelet count that often correlates with disease severity. The absence of a definitive antiviral therapy or universally available vaccine underscores the need for alternative therapeutic approaches to manage dengue symptoms and reduce complications. Medicinal plants, due to their abundance and bioactive profiles, have gained prominence in dengue management. *Carica papaya* L., a herbaceous plant commonly found in tropical regions, has been traditionally employed in the treatment of various ailments, including malaria, jaundice, and notably, dengue (3,4). The leaves of *C. papaya* have demonstrated promising hematopoietic effects, particularly in increasing platelet counts during dengue-associated thrombocytopenia. Preclinical studies have reported that aqueous extracts of papaya leaves not only improve platelet production but also exert antioxidant, analgesic, and gastroprotective effects, offering multifaceted benefits in disease conditions characterized by systemic inflammation and oxidative stress (5,6).

Despite its therapeutic potential, the formulation of papaya leaf extract into pharmaceutically stable dosage forms presents significant challenges. Plant-based preparations are often susceptible to environmental degradation, such as caking and reduced efficacy due to moisture absorption and oxidation. One promising strategy to overcome these limitations is the conversion of plant extracts into effervescent granules. These formulations improve stability, enhance palatability, and ensure accurate dosing. Effervescent systems also promote rapid disintegration and absorption, making them particularly suitable for acute conditions like dengue where timely intervention is critical (7,8). Given the growing interest in natural therapeutics and the need for stable dosage forms, this study aims to formulate effervescent granules using varying concentrations of *Carica papaya* extract and identify the most stable and effective formulation through comparative analysis.

METHODS

The study followed an experimental design aimed at preparing effervescent granules using aqueous and alcoholic extracts of *Carica papaya* leaves to assess the most stable formulation. Fresh papaya leaves were collected from the twin cities area of Pakistan and authenticated by a trained botanist. The study did not involve human or animal subjects; therefore, ethical approval and informed consent were not required. However, all procedures adhered to standard laboratory safety and research integrity protocols. Papaya leaves were gently plucked, rinsed thoroughly under tap water to remove dirt and debris, and subjected to air-drying under direct sunlight for seven consecutive days. The completely dried leaves were crushed into fine powder using a mechanical grinder and stored in an airtight container to prevent moisture absorption and degradation. This powder served as the base material for both aqueous and ethanolic extractions (9). For aqueous extraction, 50 grams of powdered leaves were soaked in 600 mL of distilled water and macerated for 12 hours with intermittent stirring. The extract was filtered using muslin cloth to remove solid residues and the clear solution was stored in a refrigerator at 4°C in an airtight container. Given the limited stability of aqueous extracts, it was recommended that this preparation be used within 24 hours (10). For alcoholic extraction, the same quantity of leaf powder (50 g) was immersed in 150 mL of ethanol in a sealed beaker and macerated over 72 hours with frequent agitation. The mixture was then decanted, filtered using Whatman filter paper, and strained through muslin cloth. The resulting filtrate was pressed to extract the remaining liquid and then stored in a sterile, airtight container. Approximately 60 mL of ethanol-based extract was obtained after processing. To prepare the effervescent granules, 40 g of citric acid, 80 g of tartaric acid, and 120 g of sodium bicarbonate were weighed accurately and passed through sieve no. 40 to ensure uniform particle size. These were thoroughly mixed in a beaker. Five grams of powdered *C. papaya* leaves were added to this blend. The mixture was then heated in a water bath while continuously stirred until granule formation was initiated through agglomeration.

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Once formed, the beaker was removed from the heat, and the granules were passed through sieve no. 20 to achieve appropriate size consistency. The granules were subsequently stored in airtight containers to prevent exposure to atmospheric moisture and degradation. Apparatus and chemicals that were used for this project is mentioned in the table 1 and 2 below:

Table 1: List of apparatus used in the preparation of effervescent granules

Sr.no.	Name	Sr.no.	Name	Sr.no.	
1	Beaker	6.	Whatman filter paper	11.	Dropper
2.	Stirrer	7.	Water bath	12.	pH paper scale
3.	Watch glass	8.	Sieve assembly	13.	Tripod stand
4.	Funnel	9.	Pestle and mortar	14	Dispensing bottle
5.	**Measuring Cylinder**	**10.**	**Weighing balance**	**15.**	**Muslin cloth**

Table 2: List of chemicals used in the preparation of effervescent granules

Sr.no.	Chemicals
1.	Papaya leaves
2.	Distilled water
3.	Ethanol
4.	Citric acid
5.	Tartaric acid
6.	Sodium bicarbonate

RESULTS

Alcoholic extract loaded effervescent granules:

The granules formulated with alcoholic extract were prepared using a base mixture of 20 g citric acid, 40 g tartaric acid, and 60 g sodium bicarbonate. After sieving and homogenizing the powders, granules were formed through wet heating and allowed to cool before being divided for extract loading. A total of 45 g of granules were obtained and passed through sieve no. 20. Half of this batch (24.7 g) was divided into two portions: Sample A was loaded with 3 g of active extract in 20 mL, while Sample B contained 2 g of extract in 15 mL. Both samples were dried in petri dishes at room temperature for 10–12 hours and stored in airtight containers. Effervescence testing in 50 mL water showed that both Sample A and Sample B exhibited a rapid reaction, initiating within 1–2 seconds and completing within 3 minutes.

Granules Stable Effervescent with the load of water-based extract:

Granules prepared via wet granulation were accurately weighed at 23.45 g. These were loaded with 20 mL of aqueous extract using a dropwise addition method. After thorough mixing and drying at ambient temperature, the granules were collected in sealed containers.

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Upon testing, these granules also produced effervescence in 50 mL of water; however, the effervescence was significantly less vigorous compared to alcohol-based formulations, indicating reduced gas release potential.

pH determination:

The pH values of the granules, assessed by dissolving 5 g in 100 mL distilled water, were within the pharmacopeial range of 4.8 to 6.2. Granules loaded with 3 g of active ingredient showed a pH of 5.0, confirming a mildly acidic nature suitable for oral dosage forms. Colorimetric paper strips and USP indicator charts were used to validate the readings.

Particle size distribution:

The sieve analysis revealed variations in particle distribution across different formulations. Granules containing 5 g of active drug showed the highest retention on sieve no. 40 (4.52 g, 68.48%), while those with 2 g and 3 g drug loadings showed major retention on sieve no. 20 (5.01 g and 5.63 g respectively, corresponding to 75.90% and 85.30%). Water-extract-based granules were predominantly retained on sieve no. 40 (5.57 g, 83.5%). No significant retention was observed on sieve no. 60 or finer grades in any formulation. Visual microscopic examination of all three types of granules confirmed predominantly spherical and irregular shapes under 4×, 10×, and 40× magnifications.

Table 3: Particle size distribution

Sr.no.	Sieve no.	Mass of powder retained (g)				Percentage retained (%)			
		5g drug	2g loaded drug	3g loaded drug	Water extract bases granules	5g drug	2g loaded drug	3g loaded drug	Water extract bases granules
1.	20	1.14	5.01	Negligible	0.27	17.27	75.90	0	4.04
2.	40	4.52	1.06	5.63	5.57	68.48	16.06	85.30	83.50
3.	60	0.02	Negligible	Trace Amount	Negligible	0.30	0	0	0
4.	80	0.21	Negligible	0.15	0.06	3.18	0	0.90	0.89
5.	100	0.34	Negligible	0.22	0.33	5.1	0	5	4.94
6.	**Collector**	**0.33**	**negligible**	**0.45**	**0.31**	**5**	**0**	**6.81**	**4.64**

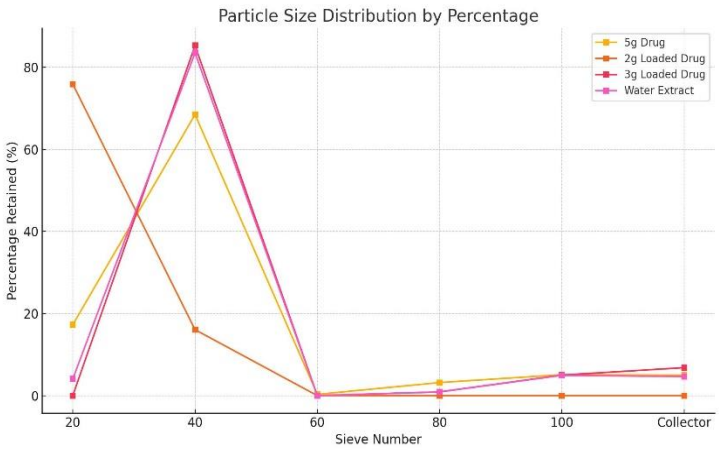

Figure 2 Particle Size Distribution by Percentage

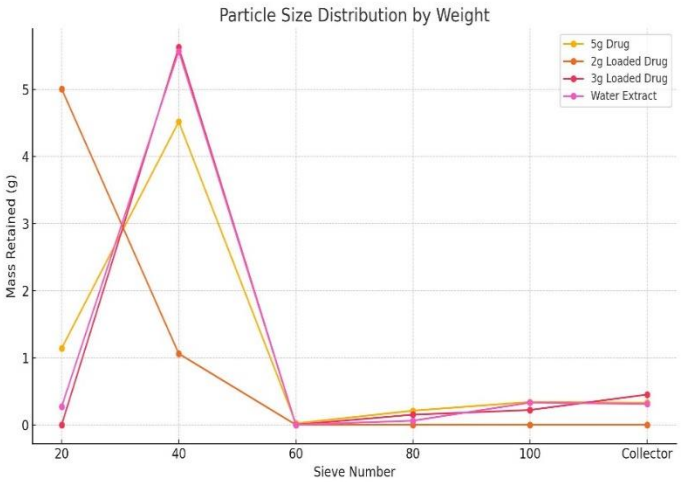

Figure 1 Particle Size Distribution by Weight

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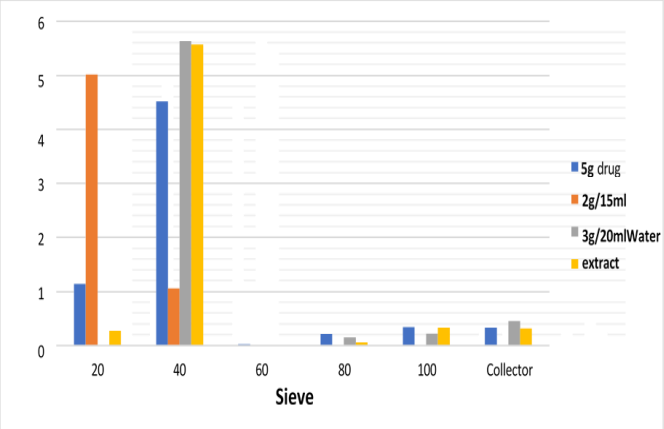

DISCUSSION

The present study explored the formulation of effervescent granules using *Carica papaya* leaf extract, aiming to enhance the stability, patient acceptability, and therapeutic utility of this traditionally used medicinal plant in managing dengue-associated thrombocytopenia. The results confirmed the potential of effervescent granules, particularly those loaded with alcoholic extracts, to demonstrate rapid effervescence, acceptable pH, and consistent particle morphology, establishing their suitability as an oral dosage form. Compared to conventional forms like tablets and capsules, effervescent formulations provided a more convenient alternative, especially for pediatric, geriatric, and bedridden patients, who often struggle with swallowing solid forms. Previous studies have documented various marketed preparations of *Carica papaya* including tablets, capsules, tea bags, and liquid extracts; however, these forms have significant drawbacks such as poor solubility, long dissolution times, and unpleasant taste profiles (11-13). In contrast, the effervescent format offered the advantages of faster disintegration, enhanced palatability, and improved absorption due to the rapid breakdown in water. Alcoholic extract-loaded granules, particularly those with 3 g/20 mL concentration, displayed optimal characteristics including rapid onset of effervescence within one second and favorable pH levels within pharmacopeial standards. This aligns with findings from earlier work where rapid-release dosage forms improved patient compliance and bioavailability of herbal constituents (14,15).

Despite the promising results, certain limitations emerged. Water-extract-based granules exhibited temperature sensitivity and poor stability beyond 24 hours, consistent with literature highlighting the perishable nature of aqueous herbal extracts and their susceptibility to microbial degradation and chemical instability (16,17). This constraint limits their application unless immediate use is ensured or further preservation techniques are applied. Moreover, the study did not explore the antimicrobial efficacy of the formulated granules, despite evidence from prior research suggesting that fresh aqueous extracts of papaya leaves may offer limited antifungal activity, particularly against select strains (18,19). A comprehensive evaluation of bioactivity post-formulation remains an unmet need and could be pursued in future investigations. Furthermore, safety concerns associated with papain-containing preparations should not be overlooked. Reports of hypersensitivity reactions, including hypotension and tachycardia following topical or ophthalmic use, warrant cautious formulation and dosage determination when developing systemic formulations. Although oral use of effervescent granules may differ in route and exposure, the potential for allergic reactions must be factored into safety profiling (20,21).

The study's main strength lies in its practical approach to improving the delivery form of a plant extract known for its hematological benefits in dengue management. The development of stable, reproducible granules with good effervescent properties reflects a translational step towards a more acceptable dosage form. Nonetheless, the absence of long-term stability data, microbiological testing, in vivo efficacy trials, and patient sensory evaluation limits the generalizability and clinical readiness of the formulation. Future research should address these gaps by incorporating stability studies over different storage conditions, evaluating bioavailability in suitable models, and assessing patient-centric outcomes like palatability and ease of use (22). Incorporation of natural preservatives or co-formulation with stabilizers could extend the shelf life of aqueous-based granules. Comparative studies between different extract types and concentrations would further refine the ideal dosage. Additionally, regulatory safety profiling for papain and other bioactive constituents should accompany any movement toward clinical application.

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CONCLUSION

This study concluded that *Carica papaya* leaf extract, when formulated into effervescent granules, offers a promising and practical approach for managing dengue-associated thrombocytopenia. Among the various formulations tested, granules prepared with alcoholic extract in the 3g/20mL ratio demonstrated the most favorable characteristics in terms of stability, pH compliance, and rapid effervescence, making them a potentially effective and patient-friendly dosage form. The findings support the use of papaya leaf extract as a supplementary therapeutic agent that may help enhance platelet recovery and reduce hospitalization burden in dengue patients. By addressing key limitations of conventional herbal preparations, this effervescent form paves the way for a more accessible and acceptable alternative in herbal drug delivery.

AUTHOR CONTRIBUTION

Author	Contribution
Sajid Raza*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sujjad Zaman	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Rubia Anwer	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Adnan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Rafi Ullah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Faqir Ullah	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Sophia Awais	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Nasir Khan	Writing - Review & Editing, Assistance with Data Curation
Waqas Ahmad Khan	Writing - Review & Editing, Assistance with Data Curation

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