Pharmacognostical Standardization, Formulation And Evaluation Of *Carica Papaya* L. Sparkling Water For Dengue Fever And Its Management

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Abstract
The incidence of Dengue fever, a mosquito-borne viral illness, has escalated in recent years across various countries including Bangladesh, Philippines, Thailand, Brazil, and India, leading to substantial morbidity and mortality. Traditional remedies utilizing *Carica papaya* leaves in fresh form have shown promise in alleviating Dengue fever symptoms, particularly thrombocytopenia, a common complication. To address issues of stability and palatability of fresh *Carica papaya* leaves a novel dosage form utilizing carbonated fresh juice of *Carica papaya* leaves is proposed. Carbonation not only preserves the bioavailability of the active constituents but also enhances patient acceptability. This innovative approach offers a convenient and effective means of delivering *Carica papaya* leaf extract for Dengue fever management, potentially improving patient outcomes worldwide. The described DNA isolation and subsequent sequencing and BLAST analysis conclusively identified the sample as *Carica papaya*, affirming the reliability and efficacy of the employed methodology for genetic analysis and species identification. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. Authentic markers of flavonol (quercetin) obtained commercially was cochromatographed. Blue brown colour zone was detected in UV derivatisation in the chromatogram which confirms the presence of flavonoid quercetin in the formulation. This novel formulation was standardized using modern sophisticated instuments like TLC, HPTLC, DNA barcoding and UV analysis. This formulation would be definitely helpful for the future mankind for better dengue management.

Keywords: *Carica papaya*, Sparkling water formulation, Dengue fever, DNA Barcoding, HPTLC Finger printing.

Introduction

Dengue, a very widespread mosquito born infectious disease caused by *Aedes aegypti* virus, has been occurring during the monsoons every year. The prevalence and incidence of dengue fever and death due to its complications have been increased drastically in these recent years in Bangladesh, Philippines, Thailand, Brazil and India (Sarker MMR, Khan F et al. 2021).

Carica papaya leaves belonging to the family Caricaceae and their juice or extracts as well as their different forms of preparations have long been used traditionally for treating dengue fever and its complications. Therapeutic applications of *Carica papaya* leaf extract in the management of the Dengue fever have been proved by many researchers. The major findings reveal that papaya leaf extract has strong medicinal properties such as antibacterial, antiviral, anti-tumor, hypoglycemic and anti-inflammatory activity. Further many clinical trials have been conducted in multi-centric, double blind, placebo controlled, randomized, prospective study to evaluate the efficacy and safety of *Carica papaya* fresh leaf juice (CPFLJ) (Singh SP, Kumar et al. 2022), as empirical therapy for thrombocytopenia associated with dengue fever without any side effect and has been found prevent the complications of thrombocytopenia. It is too difficult to always supply a fresh leaf juice of *Carica papaya* for the treatment which makes poor patients compliance and low bioavailability (Gadhwal AK, Ankit BS et al. 2016), (Hampilos K, Corn J et al. 2019).

Carbonated water (also known as Soda water, sparkling water, fizzy water, club soda, water with gas, in many places as mineral water, or especially in the United States as Seltzer water) is water containing dissolved carbon dioxide gas, either artificially injected under pressure or occurring due to natural geological process. Carbonation causes small bubbles to form, giving the water an effervescent quality. The carbonated water manufactured commercially by adding small amount of sodium and potassium salts to mimic the natural flavor and offset the acidity of introducing carbon dioxide gas. The carbon dioxide gas will be introduced naturally or by adding low 5-6 pH carbonic acid solution when dissolved in water. Carbonated water such as club soda or sparkling water is defined in US law as a food of nutritional value, even if minerals, vitamins, or artificial sweeteners have been added to it. Carbonated water may help with constipation among people who have had a stroke (Twilley, Nicola et al. 2019), (Jessica Krefting, MS et al. 2018), (Beckett J.V 1977).

Hence to overcome this problem a novel dosage form will be formulated in the form of sparkling water fresh juice of *Carica papaya* leaves for the benefit of mankind in case of dengue fever as well as thrombocytopenic patients (The BP, Ahmad NB et al. 2022), (Kature PN, Nagabhushan KH et al. 2016). This novel formulation is first of its kind to safeguard the bioavailability, nature of the papaya fresh juice without any decomposition of its phyto-constituents.

Materials and Methods

Pharmacognostical standardization

The Leaves of *Carica papaya* Linn. was collected in the month of July 2023 from Soriankuppam Village, Pondicherry, following current Good Collection Practices (cGCP). The plant material was identified and authenticated by Dr. N. Balachandran, Botanist and Ecology department at Institute Francais de Pondicherry (Verma c, Ahmad R et al. 2020).

The plant material was washed, shade dried for a day and then dried completely in an oven at 40˚C. The plant material was coarsely powdered using a rotary grinder and stored in an airtight plastic container, and then used for phytochemical screenings. Fresh leaves were used for morphological and anatomical studies (Apurva Priyadarshini, Bhuwal Ram 2018), (Yoppi Iskandar, Resmi Mustarichie 2018).

The morphological examination of leaves of *Carica papaya* was done by observing the collected sample with naked eye as well as under luminescent light for their color, size and shape. The odor and taste of the material were also observed. All these observations are noted and given in the result section (Zujar V, Mammen D et al. 2011), (Sankarganesh P, Baby Joseph et al. 2018), (Alla N, Satyanarayana T et al. 2018).

The microscopic study is done by taking transverse sections (T.S) of the plant parts stained with Phloroglucinol and Conc. HCl. The section was placed on a glass slide and few drops of glycerin is added to retain the moisture. Then the slide was covered by the coverslip and placed in the compound microscope with the help of forceps. The slide was observed under the microscope at 40X magnification (Priyadarshi A, Ram B et al. 2018), Yogiraj A, Goyal PK et al. 2014).

Proximate analysis

Physiochemical parameters such as Ash values, Extractive values and Moisture content were performed as per the customary official procedures (Iskandar Y, Mustarichie R et al. 2018), (Wadekar AB, Nimbalwar MG et al. 2021), (Yogiraj V, Goyal PK et al. 2014).

DNA bar code development

DNA was isolated from carefully selected leaf samples using a modified CTAB method. Samples (25 mg) were ground with 1 ml of CTAB buffer, incubated at 65°C for 1 hour with intermittent vortexing, and then centrifuged. The supernatant was mixed with Chloroform: Iso-amyl alcohol, followed by centrifugation to isolate the DNA-containing aqueous layer. DNA was precipitated with Isopropanol, washed with ethanol, air-dried, and re-suspended in elution buffer (50 µL) for storage at -20°C.

DNA Quality Assessment: The quantity and quality of isolated DNA were assessed using a Nano Drop 2000 spectrophotometer (Thermo, USA). The absorbance ratio at 260 nm and 280 nm (260/280) was measured to evaluate DNA purity, with a ratio falling within the range of 1.7 to 1.8 indicative of high-quality DNA devoid of protein or RNA contamination.

Primers: Universal primers targeting the RBCL gene were chosen based on previous studies for comprehensive amplification encompassing all variable regions. The primer sequences are as follows:
Forward Primer (RBCL For): 5′- ATGTCACCACAAACAGAGACTAAAGC - 3′

Reverse Primer (RBCL Rev): 5′- GTAAAATCAAGTCCACCRCG - 3′
These primers were selected to ensure robust amplification of the RBCL gene, facilitating accurate DNA barcoding analysis (Saitou N and Nei M 1987), (Felsenstein J 1985), (Tamura K, Nei M 2004), (Kumar S, Stecher G et al. 2016).

Formulation of *Carica papaya* Sparkling Water

The sample materials used in sparkling water formulation are fresh *Carica papaya* leaves and various food additives such as citric acid, sucrose and sodium benzoate. Fresh leaves of *Carica papaya* was properly sort to remove roots and undesirable particles. It was then washed in clean water weighed and crushed in a pulverize. Little water was added for easy crushing. 10 dm^3 of water was added to every 1 kg of pulverized leaves of *Carica papaya*. Muslin cloth was used for filtration and raffinate discarded. The filtered solution was allowed to stand for 2-3 hours to enable the starch settled (sediment). Mother liquor of the water soluble extract was decanted from the sediment starch. The pure *Carica papaya* extract was heated to about 70°C to eliminate microorganisms. It was then blended with food additives mentioned

above in accordance with standard set by the World Health Organization (WHO). The resulting solution is a *Carica papaya* soft drink. To carbonate the product, it was first pre-chilled in a refrigerator to lower the temperature to about 2-4°C for easy absorption of CO_2, which was then added to the *Carica papaya* leaves with the aid of carbonator. The product was then bottled and sealed immediately for freshness. The product (carbonated *Carica papaya* drink) was then analysed to determine the turbidity, ash content, pH, Titratable acidity, brix and microbial count (Abdulkarm SA, Uthman H et al. 2011), (Hemanth KJ, Hema MS et al. 2020), (Ebewele RO 1981), (Cocks LV, Rede CV 1966), (Pigan W 2012), (Horwitz W 1982).

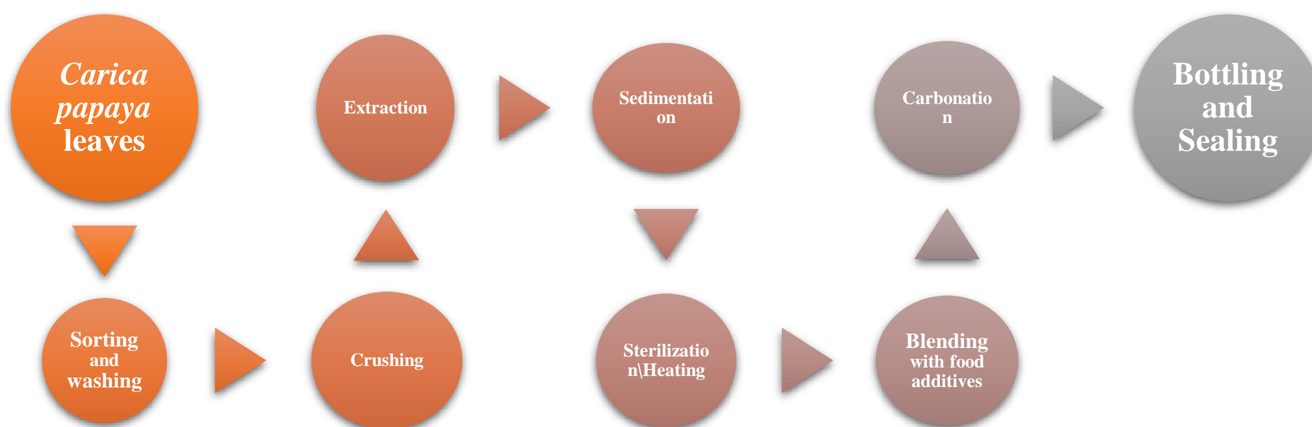


Fig 1: Production processes of Sparkling water formulation of CPFLJ

Phytochemical screening of Sparkling water formulation of CPFLJ

The sparkling water formulation of CPFLJ was subjected to qualitative phytochemical screening employing standard chemical tests to detect various classes of phytoconstituents including alkaloids, amino acids, proteins, carbohydrates, flavonoids, glycoside, saponins, sterols, tannins, phenolic and terpenoids (Zujar V, Mammen D et al. 2011), (Sankarganesh P, Baby Joseph et al. 2018), (Omidiwura BR 2017).

Determination of Total Phenolic Content (TPC)

The estimation of total phenolic content was conducted utilizing the Folin-Ciocalteau method as outlined in references. Chemicals and reagents utilized in the process included Gallic acid, Folin-Ciocalteau reagent (diluted to 1:10 ml with distilled water from a 2N stock), 7.5% Sodium carbonate, distilled water, and methanol.

To construct the standard graph for Gallic acid, 10 mg of Gallic acid was dissolved in 10 ml of methanol to achieve a 1 mg/ml solution. From this solution, 1 ml was pipetted out and diluted to 10 ml with methanol to create a 100 µg/ml Gallic acid standard solution (stock solution). Subsequently, volumes of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were transferred from the stock solution into ten separate test tubes. Distilled water was then added in volumes of 0.8, 0.6, 0.4, 0.2, and 0 ml respectively to prepare solutions containing 20, 40, 60, 80, and 100µg/ml. To each of these solutions, 5 ml of Folin-Ciocalteau reagent was added and allowed to stand for 3 minutes, followed by the addition of 4 ml of 7.5% sodium carbonate solution. A reagent blank was prepared using 1 ml of distilled water instead of Gallic acid. These test tubes were then incubated for 2 hours at room temperature shielded from strong light, resulting in the development of a dark blue coloration which was measured at 750 nm using a UV-Vis spectrophotometer.

For the preparation of sample solutions, 10 mg of the formulation was taken and made up to 10 ml with methanol to achieve a concentration of 1000 µg/ml. From this solution, 1 ml was taken and processed following the same procedure as the standard (Sulaiman CT and Balachandran I 2012).

Determination of Total Flavonoid Content (TFC)

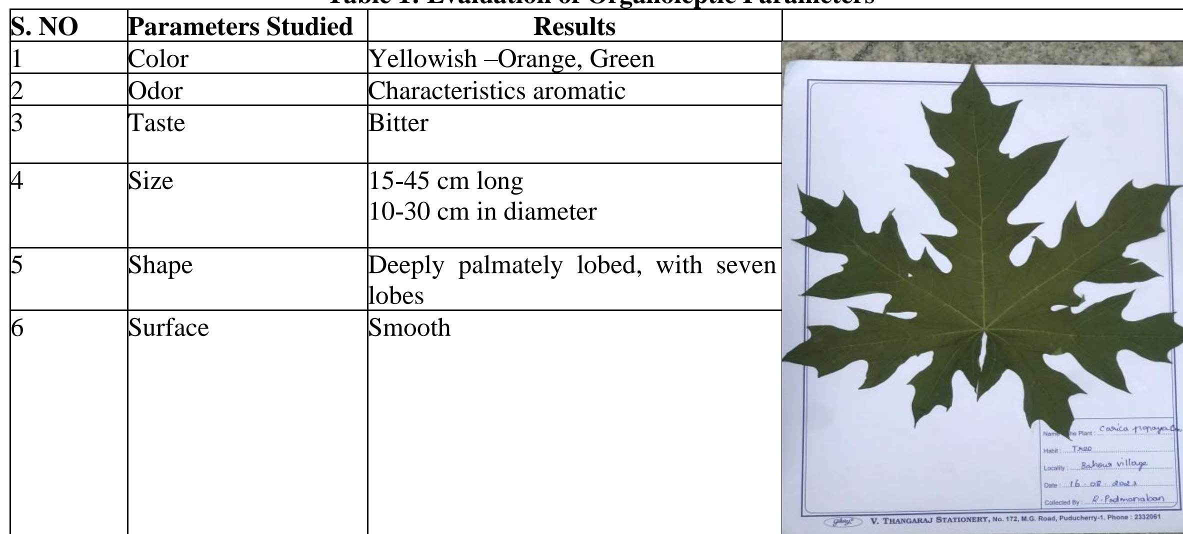
The estimation of total flavonoid content was conducted employing the Aluminum chloride colorimetric method, a well-established technique in the field. Chemicals and reagents employed in this procedure comprised Quercetin, 5% sodium nitrite, 10% Aluminum chloride, 1 M Sodium hydroxide, methanol, and distilled water. To construct the standard graph, 10 mg of Quercetin was accurately weighed and dissolved in 10 ml of methanol in a 10 ml standard flask. From this solution (1 mg/ml), 1 ml was extracted and diluted to 10 ml with methanol to obtain a 100 µg/ml Quercetin standard solution (stock solution). Subsequently, volumes of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were withdrawn from the stock solution and transferred into ten test tubes. Each tube was then supplemented with water to achieve concentrations of 20, 40, 60, 80, and 100µg/ml respectively. To each of these test tubes, 4 ml of distilled water was added followed by 0.3 ml of 5% sodium nitrite. After a 5-minute incubation period, 0.3 ml of 10% Aluminum chloride solution was introduced. At the 6th minute, 2 ml of 1 M sodium hydroxide solution was added, and the total volume was adjusted to 10 ml with distilled water. A blank solution was prepared in a similar manner, with the exclusion of

Aluminum chloride, using 0.3 ml of distilled water instead. These solutions were thoroughly mixed, and their absorbance was measured against the blank at 510 nm using a UV-VIS spectrophotometer.
For the preparation of sample solutions, 10 mg of the sample extract was weighed and dissolved in 10 ml of methanol to achieve a concentration of 1000 µg/ml. From this solution, 1 ml was extracted and processed following the same procedure as the standard. This ensured consistency and accuracy in the estimation of total flavonoid content in the samples (Omidiwura BR 2017).

TLC & HPTLC analysis of *Carica papaya* sparkling water formulation:
In this study, *Carica papaya* extracts in water and *Carica papaya* sparkling water formulations underwent comprehensive analysis using thin-layer chromatography (TLC) and High Performance Thin-Layer Chromatography (HPTLC) systems equipped with UV detection at λ_{max} 254 nm and 366 nm, alongside a natural light detection system. Aluminum plates coated with a layer of silica gel-G served as the substrate for thin-layer chromatography. The samples, consisting of water vehicle extract, were meticulously prepared and applied onto TLC plates, followed by development using a selected mobile phase are toluene: ethyl acetate: formic aid ratio of (50:40:10). The resulting spots on the TLC plates were subjected to R_f value calculation, providing crucial insights into the migration characteristics of the components within the samples (Omidiwura BR 2017), (Haldar S, Mohapatra S et al. 2020).
Central to the investigation were endeavors in standardization and the establishment of fingerprinting profiles for specific crude pharmaceuticals. Through meticulous experimentation, the HPTLC method emerged as a pivotal tool for identifying marker chemicals within the samples. Notably, the utilization of a toluene: ethyl acetate: formic acid (50:40:10) mobile phase enabled swift and accurate confirmation of Quercetin presence in the formulations. This study not only contributes to the understanding of *Carica papaya* formulations but also underscores the efficacy of HPTLC in pharmaceutical analysis, particularly in the context of standardization and compound identification (Sharma V and Janmeda P 2013).

Results and Discussion
Morphological Evaluation

Table 1: Evaluation of Organoleptic Parameters



S. NO	Parameters Studied	Results	
1	Color	Yellowish –Orange, Green	
2	Odor	Characteristics aromatic	
3	Taste	Bitter	
4	Size	15-45 cm long 10-30 cm in diameter	
5	Shape	Deeply palmately lobed, with seven lobes	
6	Surface	Smooth	

The evaluation highlights a color range from yellowish-orange to green, with a characteristic aromatic odor and bitter taste. The specimen typically measures 15-45 cm long and 10-30 cm in diameter, displaying a deeply palmately lobed structure with seven lobes.

Microscopical Studies

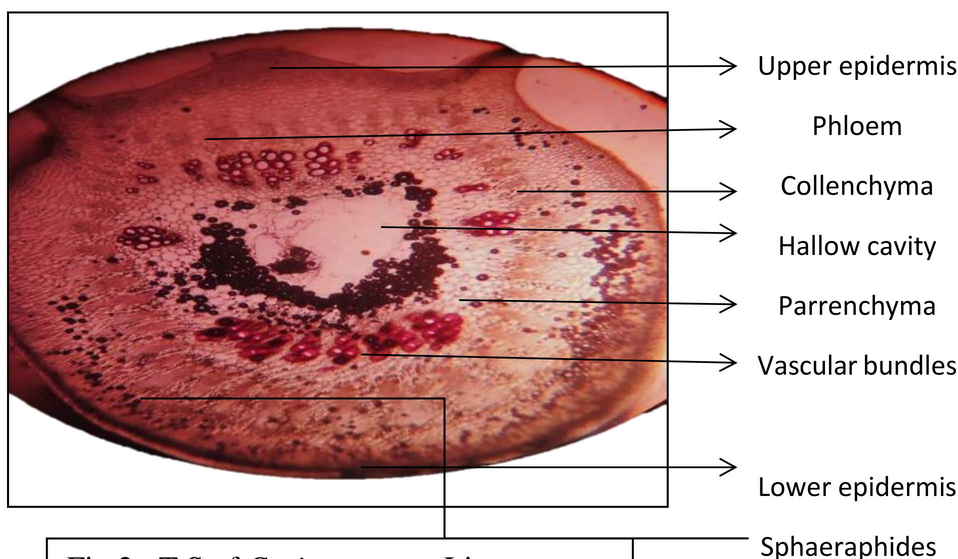


Fig 2: T.S of *Carica papaya* Linn.

The microscopic view of *Carica papaya* leaf reveals a structured composition comprising distinct cell types. The upper epidermis and lower epidermis provide protective layers, while collenchyma and parenchyma cells contribute to structural support and storage. Vascular bundles and phloem facilitate nutrient transport, while the presence of sphaeraphides suggests potential defensive mechanisms. The hollow cavity indicates a dynamic internal environment, underscoring the leaf's intricate biological functionality and adaptive strategies.

Table 2: Proximate Analysis of CPFLJ

S.NO	Parameters	Results (%w/w)
1	Water- soluble extractives	39.20±0.12
2	Alcohol-soluble extractive	17.20±1.04
3	Total Ash	17.06±0.18
4	Acid Insoluble ash	13.0±0.22
5	Water Soluble ash	3.15±0.45
6	Moisture content	5.68±0.98

Physicochemical parameters like percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined and depicted in Table 2.

DNA Bar Code Development

DNA quality determination

The quality of DNA was checked on 0.8% agarose gels.

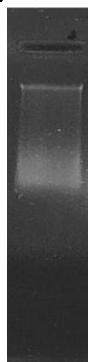


Fig 3: Genomic DNA of Sample P

PCR Condition

The amplified products were checked on 1.5% Agarose gel electrophoresis and the molecular weight was checked using molecular weight marker (100bp ladder).

1	100 bp marker
2	Sample P

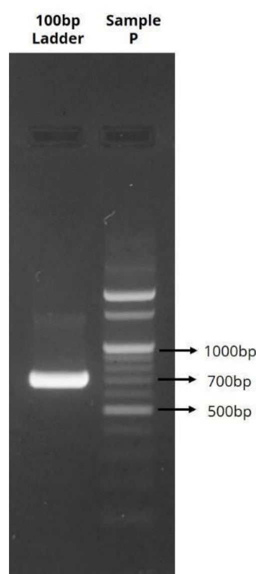


Fig 4: PCR products of RBCL region.

Sample P – Clear Chromatogram

>Sample P Partial RBCL region

GACTTATTTATACTACCTGACTATCAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCA
ACCTGG
AGTTCCACCTGAGGAAGCAGGGGCCGCGGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGG
ACCGA
TGGGCTTACCAGCCTTGATCGTTACAAAGGACGATGCTACGGCATCGAGCCCGTTCCTGGAGAAGAAAGT
CAATT
TATTGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGT
GGGTAA
TGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTAGAGGATCTGCGAATCCCTCCTGCTTATATTAAAA
CTTTCC
AGGGACCACCTCATGGTATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTATTAGGATG
TACTAT
TAAACCTAAATTGGGGTTATCCGCTAAAAACTACGGTAGAGCGGTTTATGAATGTCTACGCGGTGGACTT
GATTTT
ACCAAAGATGATGAGAATGTAAACTCCCAGCCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGA
AGCTA TTTATAAAGCACAGGCTGAAACCGGTGAAATCAAAGGGCATTATTTGAATGCTACG

Blast analysis identified sample P as *Carica papaya*

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all *100 sequences selected* — GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Carica papaya clone P14 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial ...	Carica papaya	1216	1216	99%	0.0	99.70%	1381	KX951436.1
Carica papaya ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloro...	Carica papaya	1216	1216	99%	0.0	99.70%	1323	KJ773361.1
Carica papaya ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and rb...	Carica papaya	1216	1216	99%	0.0	99.70%	1410	JX091914.1
Carica papaya ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and rb...	Carica papaya	1216	1216	99%	0.0	99.70%	1400	JX091913.1
Carica papaya chloroplast, complete genome	Carica papaya	1216	1216	99%	0.0	99.70%	160100	NC_010323.1
Carica papaya voucher PS1078MT02 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) g...	Carica papaya	1206	1206	99%	0.0	99.70%	703	GU363795.1
Vasconcellea carvalhoae voucher KUELAP227 chloroplast, complete genome	Vasconcellea car...	1205	1205	99%	0.0	99.40%	158723	NC_065369.1
Vasconcellea sprucei ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; ...	Vasconcellea spr...	1205	1205	99%	0.0	99.40%	1406	JX091960.1
Vasconcellea sphaerocarpa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, parti...	Vasconcellea sp...	1205	1205	99%	0.0	99.40%	1411	JX091959.1
Vasconcellea quercifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial c...	Vasconcellea qu...	1205	1205	99%	0.0	99.40%	1392	JX091957.1
Vasconcellea cundinamarcensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, ...	Vasconcellea cu...	1205	1205	99%	0.0	99.40%	1397	JX091955.1
Vasconcellea palandensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial ...	Vasconcellea pal...	1205	1205	99%	0.0	99.40%	1402	JX091952.1

Fig 5: Blast analysis result of RBCL region of sample

The described DNA isolation method employing a modified CTAB protocol yielded high-quality DNA from *Carica papaya* leaf samples. The subsequent quantification and quality assessment confirmed the suitability of the extracted DNA for downstream applications, with a DNA concentration of 960 ng/μL and a 260/280 ratio indicative of minimal protein or RNA contamination. Moreover, PCR amplification targeting the RBCL gene region successfully generated specific products, as evidenced by gel electrophoresis. The subsequent sequencing and BLAST analysis conclusively identified the sample as *Carica papaya*, affirming the reliability and efficacy of the employed methodology for genetic analysis and species identification. This comprehensive approach underscores the importance of robust DNA extraction techniques and molecular tools in plant biology research and biodiversity studies.

Table 3: Results of qualitative analysis of Phytochemical Constituents of Sparkling water formulation of CPFLJ

S.No	Phytocompounds	Test/Performed	Results
1	Test for Carbohydrates	Molisch test	+
		Benedict's test	+
		Barfoed's test	+
		Seliwanoff test	+
2	Test for proteins	Millon's test	+
		Xanthoprotein	+
3	Test for Amino acids	Ninhydrin's test	+
		Millon's test	+
4	Test for Steroids	Salkowski Test	-
5	Test for Flavonoids	Shinoda test	+
		Sulphuric acid test	+
6	Test for Glycosides	Keller killani test	+
7	Test for Alkaloids	Mayer's test	+
		Dragondroff's test	+
		Wagner's test	+
		Hager's test	+
8	Test for Tannins	5% FeCl3	+
		Lead acetate	+

(+) – Presence, (-) – Absence

The qualitative analysis of phytochemical constituents in sparkling water formulation of *Carica papaya* leaves (CPFLJ) reveals the presence of carbohydrates, proteins, amino acids, glycosides, alkaloids, tannins and flavonoids , as indicated by various positive tests such as Molisch, Benedict's, Millon's, Ninhydrin's, Shinoda, and Sulphuric acid tests, Keller killani, Dragondroff's, Mayer's, Hager's, Wagner's, 5% FeCl3, and Lead acetate tests. However, steroids and was not detected in the sample, as indicated by negative results in the Salkowski,. These findings suggest the potential medicinal value of *Carica papaya* leaves due to its diverse phytochemical composition.

Table 4: Results of Quantitative Analysis

S.NO	Parameters studied	Results
01	Total Phenolic content	14.45 (mg AE/gm)
02	Total Flavonoids content	5.327 (mg Quercetin equivalent/ gm)

HPTLC Photo document of Sparkling water formulation of CPFLJ

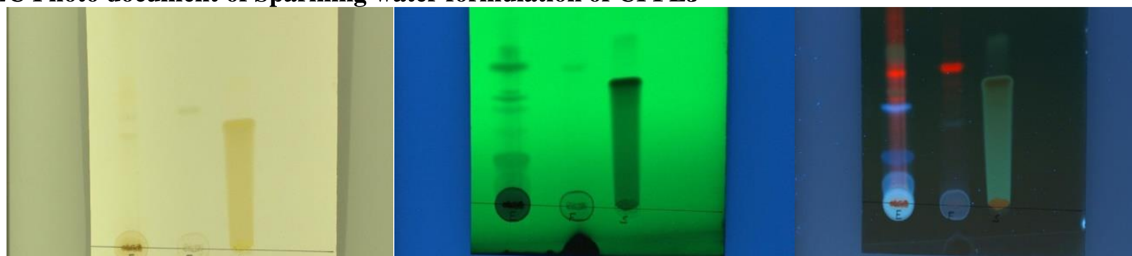


Fig: 6 a) at Natural light b)at λ_{max} 254 nm c) at λ_{max} 366 nm

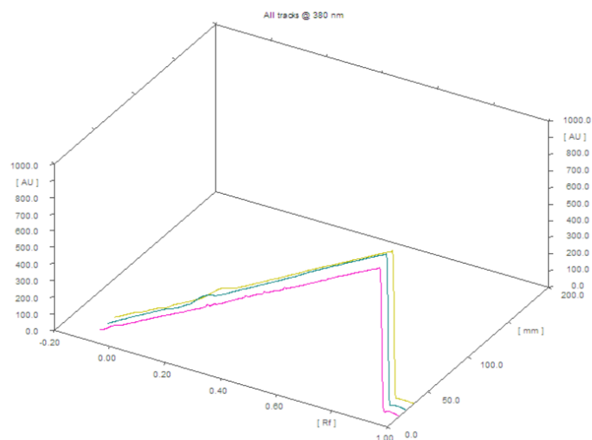


Fig 7 : 3D overley of Etract,Sparkling water formulaton of CPFLJ and Std. Quercetin

Table 5: HPTLC Profile of Extract, Sparkling water of CPFLJ, Std. Quercetin

track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	-0.07 Rf	2.4 AU	0.31 Rf	35.8 AU	28.22 %	0.32 Rf	1.3 AU	42619.1 AU	20.97 %	unknown *
1	2	0.59 Rf	67.2 AU	0.93 Rf	54.0 AU	71.78 %	0.94 Rf	4.9 AU	60623.3 AU	79.03 %	unknown *
2	1	-0.07 Rf	7.3 AU	0.93 Rf	02.7 AU	00.00 %	0.94 Rf	4.0 AU	98730.0 AU	00.00 %	unknown *
3	1	-0.07 Rf	9.0 AU	0.92 Rf	80.5 AU	00.00 %	0.93 Rf	5.1 AU	87945.3 AU	00.00 %	unknown *

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. Authentic markers of flavonol (quercetin) obtained commercially was cochromatographed. Blue brown colour zone was detected in UV derivatisation in the chromatogram which (Fig 6) confirms the presence of flavonoids. The extracts were run along with the standard polyphenols compound. HPTLC of extracts, Sparkling water of CPFLJ were found to be similar as those obtained with quercetin standards on different mobile phases. Rf values of extracts and Spakling water of CPFLJ were found to be comparable with Rf values of standards quercetin as depicted in table 5 and figure 6,7 . HPTLC chromatogram of standards quercetin, and forulation extracts with varying mobile phase is depicted in figure 6,7.

Discussion

The paper presents a comprehensive approach towards the pharmacognostical standardization, formulation, and evaluation of a novel dosage form utilizing *Carica papaya* leaves for the management of Dengue fever. The use of *Carica papaya* leaves in traditional remedies for Dengue fever has been well-established, attributed to its various medicinal properties including antibacterial, antiviral, and anti-inflammatory activities. However, challenges such as stability and palatability have limited its widespread use.

To address these challenges, we proposed a novel dosage form in the form of carbonated fresh juice of *Carica papaya* leaves. This formulation not only preserves the bioavailability of active constituents but also enhances patient acceptability due to carbonation. This innovative approach offers a convenient and effective means of delivering *Carica papaya* leaf extract for Dengue fever management, potentially improving patient outcomes.

The current research describes in detail the materials and methods employed, including pharmacognostical standardization, proximate analysis, DNA barcoding, and formulation techniques. Pharmacognostical standardization involved morphological and microscopic evaluation of *Carica papaya* leaves, ensuring the authenticity and quality of the plant material used. Proximate analysis provided insights into the chemical composition of the leaves, indicating significant levels of water-soluble and alcohol-soluble extractive values, total ash content, and moisture content.

Furthermore, DNA barcoding was conducted to authenticate the plant material and confirm its identity as *Carica papaya*. The formulation process involved the preparation of carbonated fresh juice of *Carica papaya* leaves using standard procedures, followed by phytochemical screening, determination of total phenolic and flavonoid content, and TLC & HPTLC analysis.

The results demonstrate the presence of various phytochemical constituents in the sparkling water formulation of *Carica papaya* leaves, including carbohydrates, proteins, amino acids, glycosides, alkaloids, tannins, and flavonoids. Quantitative analysis revealed significant levels of total phenolics and flavonoids, further confirming the therapeutic potential of the formulation.

The use of advanced analytical techniques such as TLC & HPTLC analysis provided valuable insights into the chemical composition of the formulation, particularly in identifying marker compounds such as quercetin. The discussion of results underscores the potential medicinal value of *Carica papaya* leaves in the management of Dengue fever, highlighting the importance of standardization and formulation techniques in developing effective herbal remedies.

Overall, the paper contributes to the growing body of literature on herbal medicine and provides valuable insights into the pharmacognostical characterization, formulation, and evaluation of *Carica papaya* leaves for Dengue fever management. The innovative dosage form described in the study holds promise for improving patient outcomes and addressing the global burden of Dengue fever.

Conclusion

In conclusion, our study presents a comprehensive approach to harnessing the medicinal properties of *Carica papaya* leaves for Dengue fever management. Through rigorous pharmacognostical standardization and innovative formulation techniques, we have developed a carbonated fresh juice dosage form that not only preserves bioavailability but also enhances patient acceptability, offering a promising solution to address the challenges in Dengue fever treatment. This research contributes significant insights into herbal medicine, emphasizing the potential of *Carica papaya* leaves to alleviate the global burden of Dengue fever.

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