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The Evaluation and Characterization of *Citrus micrantha* Pectin as a Suspending Agent in Paracetamol Suspension

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Abstract: This study investigated the potential of *Citrus micrantha* pectin as a natural suspending agent in paracetamol suspension. Paracetamol suspensions are commonly used for pain relief, but they often face challenges in terms of sedimentation and uneven drug distribution. Traditional synthetic suspending agents, while effective, raised concerns about biocompatibility and environmental impact. This research aimed to evaluate the physicochemical properties of pectin extracted from *C. micrantha*, a sour citrus fruit rich in pectin, which may be used as a natural suspending agent in paracetamol suspension.

The study utilized various concentrations of *C. micrantha* pectin (1%, 2%, and 3%) in paracetamol suspension, assessing parameters of a suspension formulation such as pH, viscosity, sedimentation rate, redispersibility, and flowability. Results showed that *C. micrantha* pectin demonstrated suitable functionality as a suspending agent particularly at 2% and 3% concentrations of the formulated paracetamol suspension, outerperforming the commercial pectin in several parameters such as sedimentation rate. At 2 and 3% concentration of *C. micrantha* pectin, the formulated paracetamol suspension exhibited slower sedimentation rates and higher volumes or ration maintaining stability compared to the commercially available pectin. A slower sedimentation rate allows uniform dosing and prevention of flocculation in suspensions. In addition, in the redispersibility test, *C. micrantha* showed pronounced advantage over the commercial pectin as it only required 2-4 inversions regardless of the concentration over the course of 4-weeks, whereas the commercial pectin warrant more agitation particularly after a period of 2 weeks. This indicates that *C. micrantha* pectin as a suspending agent has better sedimentation activity and redispersibility. A suspension that is easy to redisperse means better stability and prevents from flocculation and caking (Da Silva et al. 2022).

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I. INTRODUCTION

Citrus micrantha (Rutaceae), a small, sour citrus fruit known locally in the Philippines as "biasong (*C. micrantha var. micrantha*)" or "samuyaw (*C. micrantha var. microcarpa*)", are commonly found in the islands of Cebu. This fruit is rich in pectin, and its potential for use in pharmaceuticals has not been fully explored yet, given it has mucoadhesive properties and biocompatibility. It is hypothesized that it could serve as an effective suspending agent, as such pectin from *C. sinensis* which belongs to the same family, Rutaceae, displayed suspending characteristics at concentrations as high as 3.0% (Nwakile et al. 2023). This study was designed to evaluate whether pectin extracted from *C. micrantha* can serve as an effective suspending agent in a paracetamol suspension, comparing its performance to commercially available pectin, through the following evaluation parameters: pH level, sedimentation rate, redispersibility, viscosity and flowability.

Pectin has been used successfully for many years as a thickening agent, a gelling agent, and a colloidal stabilizer in the food and beverage sector. Pectin, given its mucoadhesive properties and biocompatibility, has already gained popularity in studies used as a suspending agent. In a review study by Ubieko and Onugwu et.al (2023), they reviewed that pectin extracted from *Citrus sinensis* was an effective suspending agent for oral liquid dosage forms by using the extracted pectin to formulate an oral suspension powder of artemether and lumefantrine (Attama et.al 2022). Pectin from the Rutaceae family with the genus *Citrus*, are the most economically important part of this family; among this that are proven to be effective as suspending agent are *C. sinensis* and *C. maxima* (Ubieko et al. 2023). Pectin is a group of cell wall polysaccharides or glycan domains. It is primarily composed of around 70% D-galacturonic acid residues (GalA)

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connected at a-1,4 linkages, along with various neutral sugars like arabinose, rhamnose, and galactose. The GalA units linked at a-1,4 positions can undergo methyl esterification or acetylation (Noreen et.al 2017; Picot-Allain et.al 2020). Polysaccharides, such as pectic substances, are typically produced in the golgi apparatus of the cell wall, involving a complex process. The synthesis of pectin requires several specific enzymes acting as catalysts to create various glycosidic linkages and modify glycosyl residues within the pectic chains. Enzymes involved in this process include acetyltransferase, arabinosyltransferase, glycosyltransferase, galacturonosyltransferase, glucuronosyltransferase, methyltransferase, and xylosyltransferase (Dranca and Oroian 2018).

Table 1 Formula for Preparation of Paracetamol Suspension.

Ingredient	**Quantities**
Paracetamol Powder	5.0 g
Benzoic Acid (0.1% w/v)	0.1 g
Suspending Agent (*C. micrantha*/ Commercial) Pectin (1%, 2,% 3% w/v)	1.0, 2.0, 3.0 g
Distilled Water	100.0 mL

Table 2 Percentage Yield Using Triplicate Analysis of the Pectin Extract of *C. micrantha* Peels.

Trial	**Weight of Dried *C. micrantha* Rinds**	**Weight of *C. micrantha* Pectin Extracted**	**Percentage Yield**
1	30g	3.34	**11.13**
2	30g	1.56	**5.2**
3	30g	2.10	**7.0**
Mean ± SD			**7.78% ± 3.04%**

Table 3 FTIR Peaks of *C. micrantha* Pectin

***Citrus micrantha* Pectin (cm^{-1})**	**Functional Groups Found**
3345 – O–H stretch (alcohol/phenol)	Consistent (hydroxyl groups in pectin)
2920 – C–H stretch (alkane)	Consistent (aliphatic chains)
1742 – C=O stretch (ester/ketone)	Consistent (ester groups in pectin)
1630 – C=C or N–H bending	Slight deviation; likely COO^- (pectin-related)
	Common in carbohydrates
1150–1050 – C–O stretch (ether/alcohol)	Generally consistent; pectin contains both types
1050 – C–O stretch	Consistent (confirms polysaccharide nature of pectin)

The Rutaceae family, comprising around 154 genera and roughly 2,100 species, is known for its variety of flowers and fruits. Within this family, the *Citrus* genus holds the greatest economic significance, including fruits like oranges, mandarins, and lemons (Appelhans et. al 2021). Citrus fruits are rich in carotenoids and apocarotenoids, which are responsible for their distinct colors and fragrances. Polysaccharides such as pectin have long been utilized in the food and pharmaceutical industry in various ways including as suspending agents. As an example, the pectin from *Musa paradisiaca* was extracted and evaluated, exhibiting suspending properties at concentrations 1% w/v and 2% w/v (Owusu et al. 2023). Their accessibility and availability bring forth an inexpensive and readily obtainable source of natural excipients which offers comparable performance as synthetic ones hence why it is increasingly utilized in the pharmaceutical industry.

This study therefore seeks to evaluate the suspending properties of pectin from biasong pectin. Its suspending properties if found comparable to standard suspending agents can serve as a good option in pharmaceutical preparation of sus- pensions and ultimately reduce the cost of producing liquid pharmaceutical dosage forms for local manufacturers in the Philippines.

II. MATERIALS AND METHODS

A. *Materials. Citrus micrantha:*

Pectin, benzoic acid (JBL), commercial pectin (Sigma Aldrich, USA), paracetamol powder (FOREVER PHARMACY; purity- ≥99%), ethanol (90%), and distilled water. All other reagents used were of analytical grade.

B. *Method:*

➢ *Sample Collection and Drying:*

Citrus micrantha (biasong) was collected from a single tree in barangay Biasong, Lopez Jaena, Misamis Occidental and then peeled with sanitized knives. The collected peels were washed with running water to wash off any impurities. The collected peels were air-dried in a shaded, well-ventilated area specifically at 25-30 degrees celsius for 10-14 days to reduce moisture content without degrading the phytochemical constituents. Once dried, it was then grounded into fine powder (0.25mm) using a mechanical grinder, specifically JML Nutri Blitzer blender, and must be able to pass through a 60-mesh sieve for easier extraction of pectin (Yapo et al. 2007).

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➢ *Extraction of Pectin:*

Pectin was extracted from *C. micrantha* fruit peels using 0.05 M HCl (10 mL per gram of peel) to break down cell structures (Yapo et al., 2007). The powdered peel was boiled in the solution for 1–2 hours at 80–100°C, then filtered through sanitized cheesecloth under reduced pressure. A 2:3 ratio of 90% ethanol was added to precipitate the pectin (Hani et al., 2012). The pectin was collected, washed with distilled water, and dried at 70°C for 4 hours using a microwave oven (Huyen et al., 2015). The final powdered pectin was stored in airtight polyethylene bags inside a desiccator until use as a suspending agent.

C. Characterization of Pectin:

➢ *Pectin Yield:*

The weight of the extracted *C. micrantha* pectin and the powdered peel was taken and the calculation of the percentage yield followed the formula below using triplicate analysis of 10-gram sample of the powdered *C. micrantha* pectin (Owusu et al. 2022).

$$Pectin\ Percentage\ Yield\ \left(\frac{w}{w}\right) = \frac{Weight\ of\ Extracted\ Pectin}{Weight\ of\ Pulverized\ Peel}\ x\ 100$$

Fig 1 Extracted Pectin of *C. micrantha*

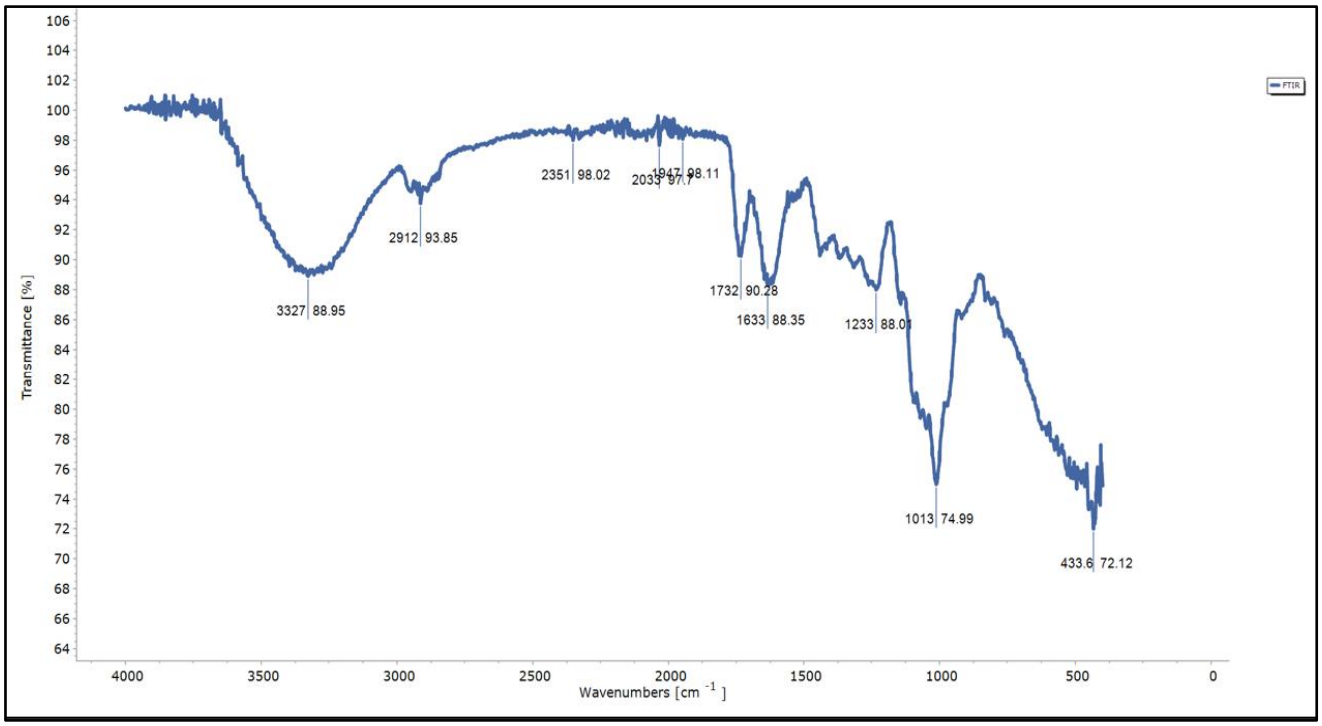

Fig 2 FTIR Spectra of *C. micrantha* Pectin.

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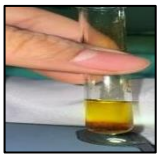
Table 4 Identification of *C. micrantha* Pectin.

Test	Observation	Inference
Pectin solution + heating and cooling	A stiff gel was formed on cooling	Pectin present
Pectin solution + ethanol	A yellowish, gelatinous precipitate was formed	Pectin present
Pectin solution + NaOH (2M)	A translucent gel was formed	Pectin present
Pectin gel + HCl (0.05M) + boiling	White cotton-like precipitates were formed	Pectin present

Table 5 Phytochemical Constituents in Extracted Pectin.

Phytochemical Test	Visible Results		Interpretation
Saponins: Foam Test		Persistent foam lasted until 10 minutes	++
Flavonoids: Ammonia Test		Presence of yellow coloration that disappears upon standing	+++
Tannins: Ferric Chloride Test		Presence of blue-black coloration	+++ (Blue black indicates Hydrolysabe Tannins)
Glycosides: Keller- Killiani Test		Formation of brown-ring at the interface	++

➢ *Fourier Transformed Infrared (FTIR) Spectroscopy:*

FTIR analysis of *C. micrantha* pectin was conducted at MSU-IIT's Center for Sustainable Polymers using a Bruker Alpha II FTIR spectrophotometer (400–2000 cm^{-1}). The degree of esterification (DE) was calculated by analyzing peak areas of free and esterified carboxyl groups, following the method of Owusu et al. (2024).

$$R = \frac{A\ (esterified\ C=O)}{A\ (esterified\ C=O) + A\ (carboxyl\ group)}\ x\ 100$$

$$DE = 124.7\ x\ R + 2.2013$$

➢ *Test for Saponins*:

Foam test The powdered pectin was boiled with distilled water, filtered hot, and cooled. A 1:1 mixture of filtrate and distilled water was shaken; persistent foam for 10 minutes confirmed the presence of saponins.

➢ *Test for Tannin:*

Ferric chloride test. About 0.5g of pectin was boiled in 10mL distilled water, filtered, and treated with 0.1% ferric chloride. A brownish green or blue-black color indicated the presence of tannins (Ayoola et al., 2008).

➢ *Test for Glycosides:*

Keller- Killiani test. About 0.5g of extract was mixed with glacial acetic acid and 1% $FeCl_3$, then layered with concentrated H_2SO_4. A brown ring at the interface, along with violet and greenish rings, indicated the presence of deoxy sugars (Owusu et al., 2022).

➢ *Test for Flavonoids:*

Ammonia test. Aqueous pectin extract was mixed with dilute ammonia and concentrated H_2SO_4. A yellow color that faded on standing indicated flavonoids (Ayoola et al., 2008).

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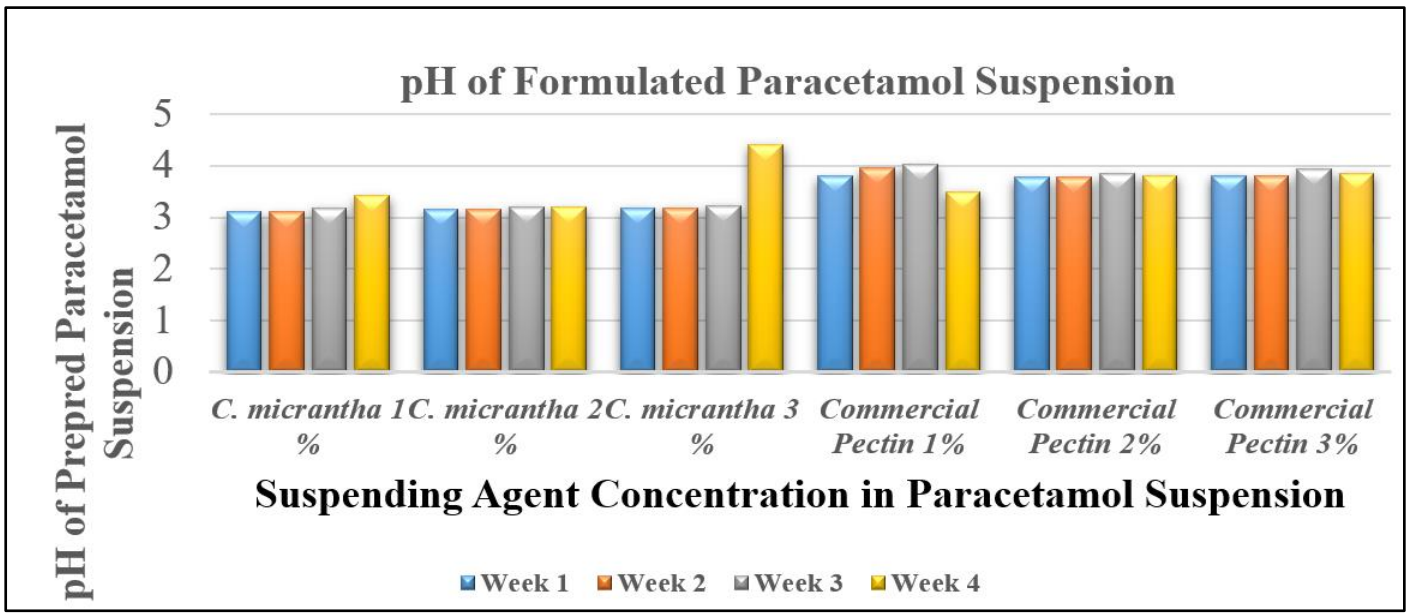

Fig 3 pH of Commercial and *C. micrantha* Pectin Suspensions

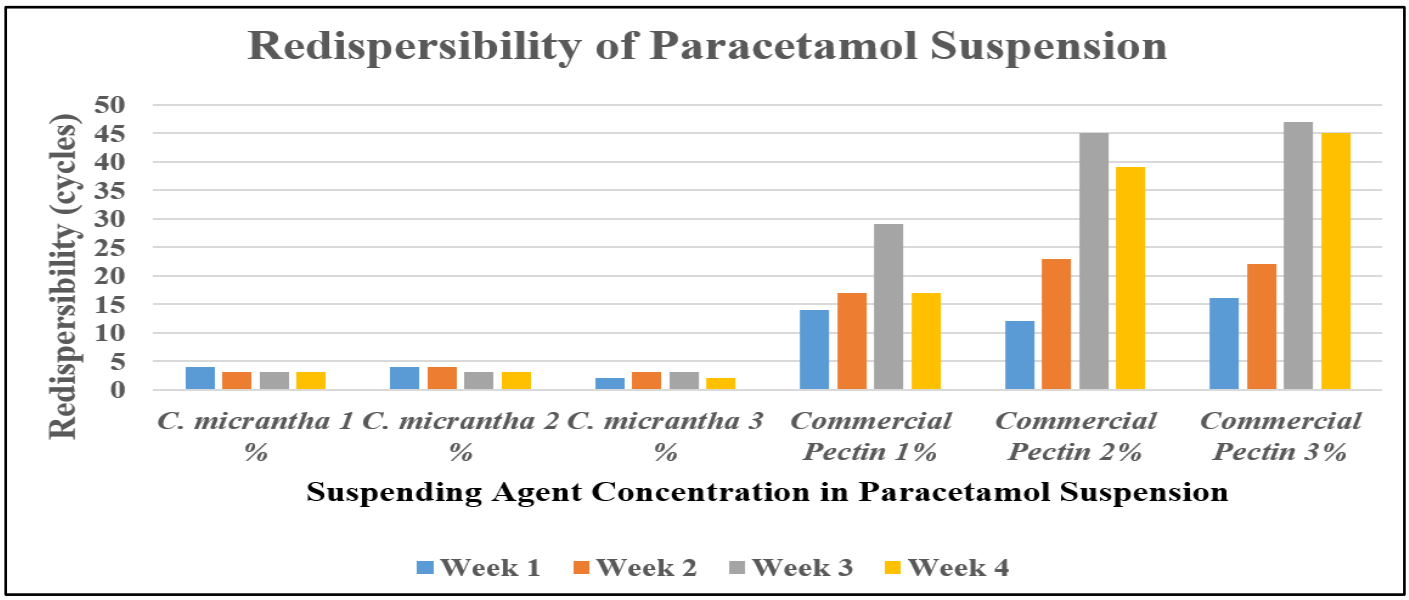

Fig 4 Redispersibility of Commercial and *C. micrantha* Pectin Suspensions

A. Preparation of Paracetamol Suspension:

The paracetamol suspension (2.4% w/v) was created using pectin concentrations of 1.0% w/v, 2.0%, w/v, and 3.0% w/v, with 0.10% benzoic acid as preservative. The suspensions was prepared by doubling the bulk of the powders in a mortar. A slurry was then formed with distilled water, followed by the addition of the preservatives. The necessary amount of distilled water was added, and the mixture was then homogenized at 2,000 rpm using an electronic homogenizer for 3 minutes to achieve a uniform consistency. Finally, the prepared suspension was transferred into an amber bottle. This method was consistently applied for all suspensions (Owusu et al., 2022).

B. Quality Control Tests on Formulated Suspensions pH.:

The formulations were carefully placed into 50ml beakers and mixed thoroughly to ensure uniform distribution before the electrodes of the pH meter are immersed. Measurements were then taken in triplicate and their means and standard deviations were recorded (Owusu et al. 2022).

C. Sedimentation Rate and Volume:

Sedimentation rate was assessed by recording sediment height every 10 minutes for 60 minutes. Sedimentation volume was measured weekly for four weeks using 50 mL of suspension and calculated using F = Vu/Vo, where higher values indicate better physical stability. All measurements were done in triplicate, and mean values were recorded (Owusu et al., 2022).

D. Viscosity and Flow Rate:

Viscosity was measured using a digital viscometer with RV spindle No. 2. Flow rate was determined by timing how long 10 mL of suspension passed through a 10 mL pipette.

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The test was repeated three times, and the average and standard deviation were recorded. Flow rate was calculated as f = volume/time (Owusu et al., 2024).

E. Redispersibility:

Redispersibility was qualitatively evaluated by placing portions of the suspension into 50ml measuring cylinders and storing them in a refrigerator for 24 hours. Suspensions with varying concentrations of *C. micrantha* pectin and commercial pectin were tested weekly over four weeks. Redispersibility was assesed by inverting the cylinders 180◦, and the number of strokes needed to fully resuspend the contents was recorded. Measurements were done in triplicate (Owusu et al. 2022).

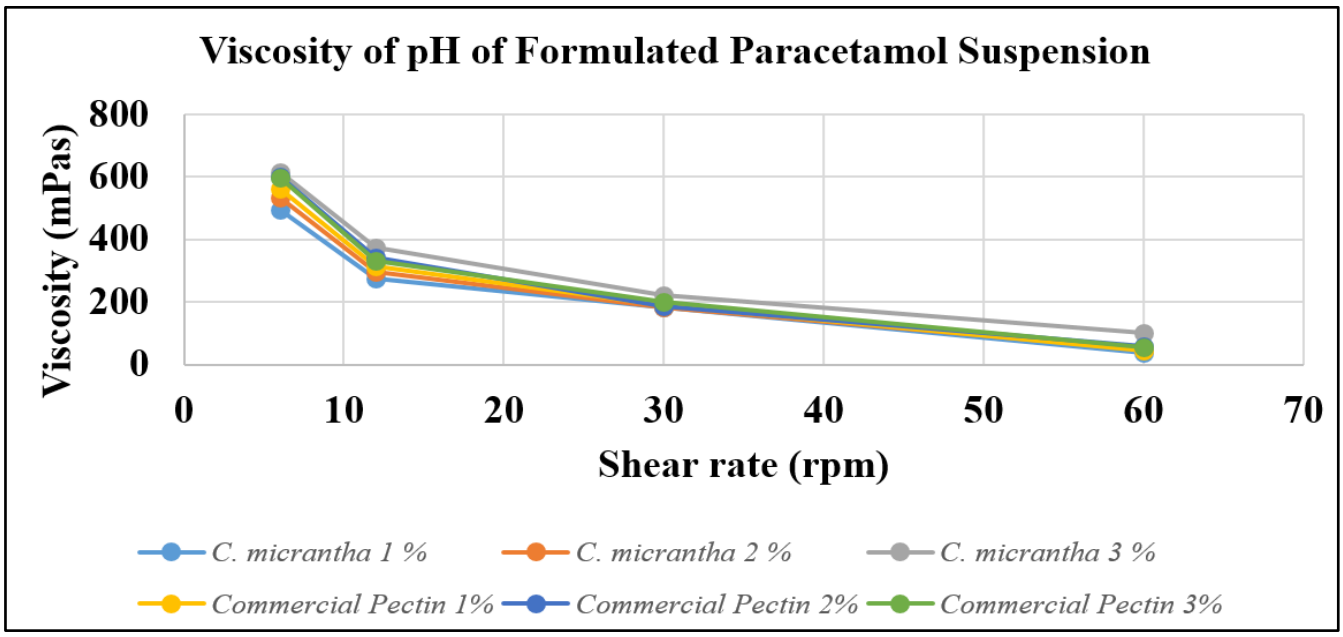

Fig 5 Viscosity of Commercial and *C. micrantha* Pectin Suspensions

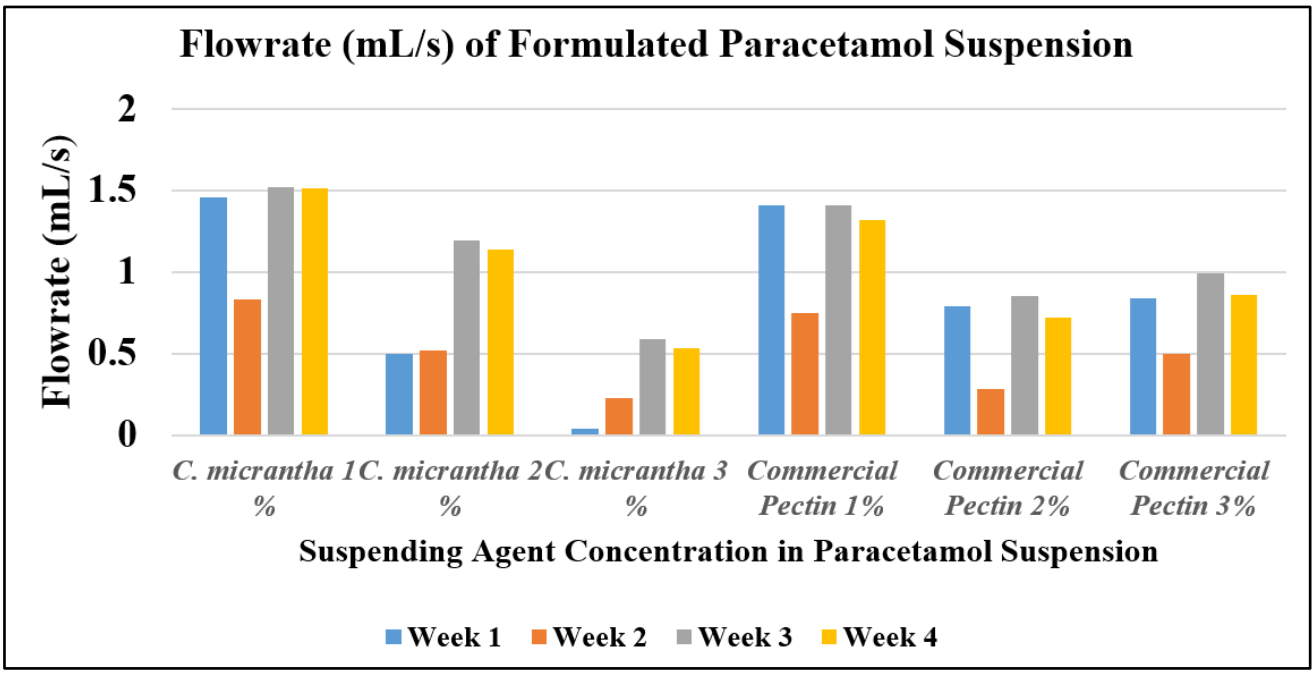

Fig 6 Flowrate of Commercial and *C. micrantha* Pectin Suspensions

III. RESULTS AND DISCUSSIONS

A. Characterization of Extracted Pectin:

The extracted pectin from *C. micrantha* peels initially has a dark to light brown/green jelly or gel-like feel before it was dried. Upon drying it in the oven for 4-6 hours it then have a powdery fixed texture.

Table 2 shows the individual yield obtained from each trial and the average/mean of the extracted pectin of *C. micrantha*.The average percentage yield was based on the triplicate analysis of 30-gram sample and is 7.78% ± 3.04%. This shows that the data obtained was of accepted range meaning the method performed can be considered accurate.

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The results of the phytochemical confirmatory test for the presence of pectin in *C. micrantha* peels are shown in Table 5. The tests were performed in IIT-CSM laboratory. All necessary phytochemicals for the performance of suspension was present.

The FTIR (Fourier Transform Infrared Spectroscopy) analysis of *Citrus micrantha* pectin reveals characteristic peaks that confirm the presence of key functional groups typically found in pectin. A strong broad absorption peak at 3345 cm^{-1} indicates O–H stretching vibrations, which is consistent with the presence of hydroxyl groups commonly found in the polysaccharide structure of pectin. The peak at 2920 cm^{-1} corresponds to C–H stretching in alkanes, suggesting the presence of aliphatic chains, which are also integral to pectin's molecular backbone. A notable absorption at 1742 cm^{-1} is attributed to C=O stretching of ester or ketone groups, further confirming the presence of esterified galacturonic acid residues in the pectin molecule.

Additionally, the peak observed at 1630 cm^{-1} may be due to C=C stretching or N–H bending; however, in the context of pectin, this slight deviation is likely associated with carboxylate (COO^-) groups, which are typical of partially de-esterified pectin. The broad region between 1150–1050 cm^{-1} is indicative of C–O stretching vibrations, which are common in carbohydrates and align well with the complex polysaccharide nature of pectin. Lastly, the distinct peak at 1050 cm^{-1} further supports the presence of C–O bonds, confirming the polysaccharide backbone structure of *C. micrantha* pectin. Collectively, these FTIR findings validate the successful extraction and identification of pectin, aligning with known spectral characteristics of commercial and natural pectin sources.

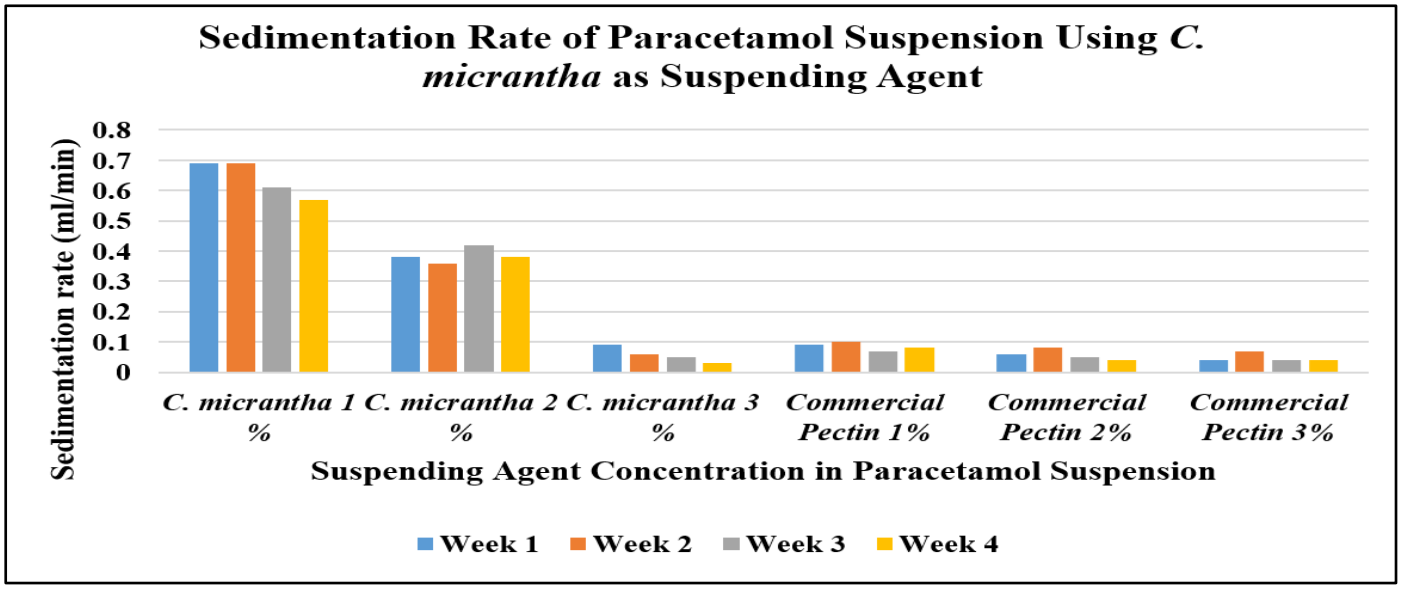

Fig 7 Sedimentation Rate of Commercial and *C. micrantha* Pectin Suspensions

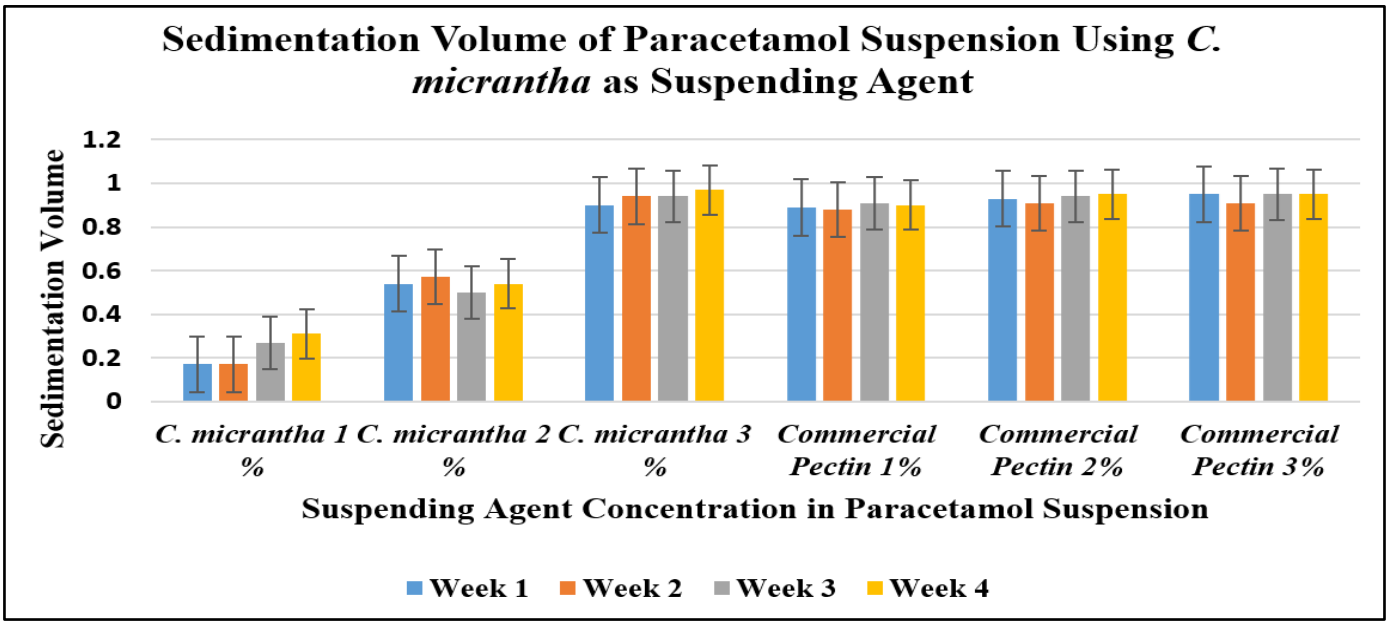

Fig 8 Sedimentation Volume of Commercial and *C. micrantha* Pectin Suspensions

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The degree of esterification (DE) of *C. micrantha* pectin was calculated based on the FTIR spectrum using absorbance values derived from peaks at 1742 cm^{-1} (esterified C=O) and 1630 cm^{-1} (carboxyl group). The transmittance values at these peaks were approximately 88% and 85%**,** respectively. Using the equation: A= log (100/T). The absorbance values were estimated as $A_{1742} = \log_{10}(100/88) \approx 0.056$ and $A_{1630} = \log_{10}(100/85) \approx 0.071$.

Determining the R:

$$R = \frac{A_{1742}}{A_{1742}+A_{1630}}\ x\ 100 \qquad (1)`$$

$$R = \frac{0.056}{0.056 + 0.071}\ x\ 100 \qquad (1)$$

$$R \approx \mathbf{44.09} \qquad (1)$$

Then applying the DE formula:

$$DE = 124.7\ x\ \mathbf{44.09} + 2.2013$$

$$DE = \mathbf{57.18}\%$$

According to Sirin 2021, if the degree of esterification in the pectin molecule is more than 50%, it is called high methoxyl pectin (HMP), and if it is below 50%, it is called low methoxyl pectin (LMP), thus making *C. micrantha* pectin with a DE of 57.18% a high methoxyl pectin (HMP). High methoxyl pectin (HMP) indicates that more than 50% of the galacturonic acid units in the pectin are methyl-esterified. This type of pectin requires a high sugar concentration and a low pH (typically between 2.8 and 3.5) to form a gel.

The paracetamol suspensions were prepared using pectin concentrations of 1.0% w/v, 2.0% w/v, and 3.0% w/v. The formula for formulating the suspensions is given in Table 8. Only the synthetic pectin and *C. micrantha* pectin (suspending agent) concentrations were varied in the formula. The suspensions were prepared using the method of doubling the bulk for powders in a mortar with levigation techniques. The required volume of water was then added, and the resultant mix was homogenised at 2000rpm with an electronic homogeniser (Sonifer) for 3 minutes to obtain a homogenous mixture. Figure 13 shows the visual representation of the formulated paracetamol suspensions.

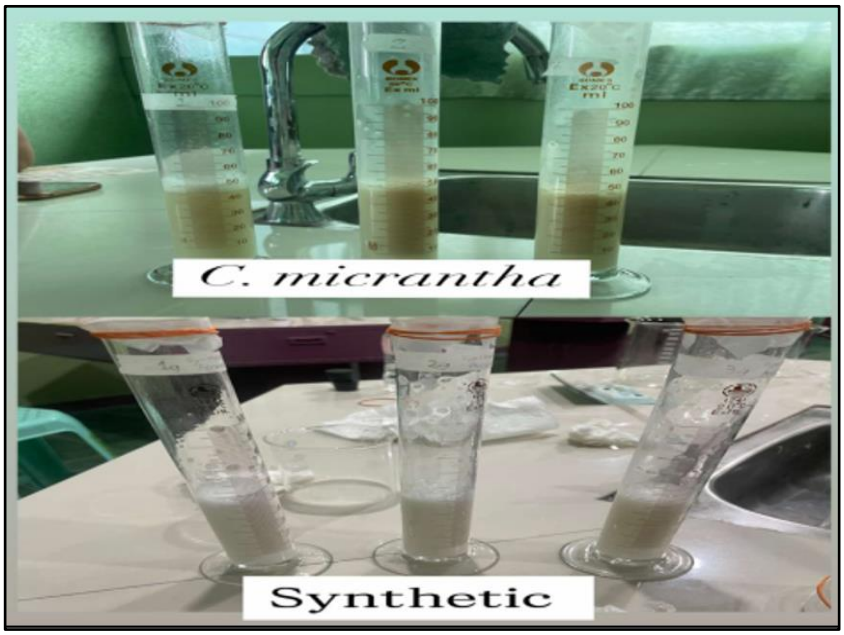

Fig 8 Physical Appearance of the Paracetamol Suspension Using *C. micrantha* Pectin and Commercially Available Pectin

B. Evaluation of Formulated Suspensions:

➢ *Data Analysis Tools:*

All analyses were performed in R (v4.2 +) with core and mixed-model packages (e.g.**,** stats**,** lme4**,** lmerTest**,** emmeans**,** broom.mixed**,** performance), applying two-tailed tests at $\alpha = 0.05$. Separate two-way Type III ANOVAs examined how pectin source (natural vs. commercial) and concentration (1 %, 2 %, 3 % w/v) affected pH, viscosity, and flow rate. Sedimentation height over 60 min was assessed with a three-factor repeated-measures ANOVA (Time within subjects; Source × Concentration between subjects). Weekly sedimentation-volume ratios across four weeks were modeled with a linear mixed-effects model that included fixed effects for Source, Concentration, Week, and their interactions plus a random trial intercept. Redispersibility (number of inversions required to resuspend) was evaluated via a Poisson generalized linear mixed model using the same fixed-effects structure and random intercept.

➢ *PH, Viscosity and Flowrate:*

As seen in the graphical data, the pH of *C. micrantha* pectin and the commercial pectin is quite comparable with one another. Data shows that within the span of 4 weeks, the pH of the different formulations remained constant which suggest that there is stability of pH. The pH of between 3.21 to 3.5 of

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the suspension with *C. micrantha* pectin can also be expected since most citrus fruits have a low pH e.g. lime with a pH of 2.16 and lemon with a pH of 2.87 (Ogundele et al. 2021). According to the study of Vardan 2024, in the study of influence of stability of pharmaceutical compounds, a low pH is able to prevent and slow certain degradation reactions which is ideal for the stability of pharmaceutical products.

Across all tests, *Citrus micrantha* (biasong) pectin behaved much like commercial pectin, with three main nuances: (1) it consistently lowered suspension pH by about 0.6 units at every 1 – 3 % loading, while neither source showed pH shifts within its own concentration range; (2) viscosity did not differ meaningfully between sources or concentrations—every pairwise comparison fell within confidence intervals that spanned zero; and (3) flow-rate slowed predictably as pectin concentration rose for both sources, yet the only cross-source gap appeared at 3 %, where *C. micrantha* suspensions poured slightly faster than commercial ones. Taken together, these findings show that the natural biasong pectin reliably acidifies formulations, delivers rheology equivalent to commercial material, and mirrors the expected concentration-driven reduction in pourability, diverging from its commercial counterpart only in a modest flow advantage at the highest loading.

- *Sedimentation Kinetics:*

The statistical analyses of sedimentation behavior confirm that both pectin source and concentration significantly influence the physical stability of paracetamol suspensions. *C. micrantha* pectin at 1 % concentration exhibited significantly faster settling, lower sedimentation volumes, and steeper sedimentation curves than all other formulations, indicating poor initial suspension stability. However, increasing the concentration to 2 % and 3 % greatly enhanced performance—slowing sedimentation rates and boosting sedimentation volume ratios to levels comparable with or even surpassing commercial pectin, especially by Week 4. Notably, the stability improvements were more pronounced over time for the natural pectin, suggesting a delayed but effective stabilization effect. In contrast, commercial pectin maintained consistently high suspension stability across all concentrations. Overall, these findings establish that while *C. micrantha* pectin underperforms at low concentration, it matches or exceeds commercial performance at higher loadings, particularly at 3 %, making it a viable natural alternative as a suspending agent when used at sufficient levels.

- *Redispersibility:*

The statistical analysis of redispersibility clearly demonstrates that *C. micrantha* pectin offers a significant advantage over commercial pectin in terms of ease of re-suspension. Regardless of concentration or storage duration, natural pectin formulations consistently required only 2–4 gentle inversions to fully redisperse, and this effort slightly decreased over time. In stark contrast, commercial pectin suspensions required dramatically more inversions—often 10 to 20 times greater—peaking at over 40 inversions by Week 3, especially at higher concentrations. These findings indicate that *C. micrantha* pectin resists irreversible sedimentation ("caking") far better than commercial pectin, making it a superior suspending agent in terms of long-term redispersibility and user convenience.

IV. CONCLUSION

Pectin isolated from *Citrus micrantha* peel (7.78 ± 3.04 % yield) is chemically verified by phytochemical screening, FT-IR, and a high degree of esterification (57.18 %), classifying it as high-methoxyl pectin. Formulated at 2 %–3 % w/v in paracetamol suspensions, this natural pectin:

- Maintains a stable, drug-compatible pH for at least four weeks;
- Provides viscosity and flow profiles indistinguishable from commercial pectin, ensuring easy pourability while limiting rapid settling;
- Slows sedimentation and sustains higher sedimentation-volume ratios than commercial pectin, especially at 3 %;
- Requires only 2–4 gentle inversions to redisperse, versus 10- to 40-plus strokes for commercial preparations, demonstrating markedly better long-term reversibility.
- Taken together, these results show that *C. micrantha* pectin not only matches but in key aspects (sedimentation control and redispersibility) surpasses commercial pectin when used at ≥ 2 % w/v. The material therefore represents a viable—and potentially superior—natural suspending agent for oral pharmaceutical suspensions.

RECOMMENDATIONS

This study confirms that *C. micrantha* pectin is a viable natural suspending agent for paracetamol suspension. However, due to its slightly lower pH, the use of pH modifiers is recommended to align with physiologic targets. For broader application, further studies are advised, including extended stability testing under various conditions, as well as toxicological and biocompatibility evaluations to confirm safety. Lastly, the potential of *C. micrantha* pectin should be explored in other liquid formulations with different active ingredients to fully assess its versatility and value in pharmaceutical suspensions.

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