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Original Article

Evaluation of *Psidium guajava* Leaf Powder and DFDBA with Injectable Platelet-Rich Fibrin for Periodontal Regeneration: A Cone-Beam Computed Tomography-Based Clinical Study

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ABSTRACT

This trial was designed to assess and compare the clinical and radiographic effects of using *Psidium guajava* leaf powder together with injectable platelet-rich fibrin (iPRF) against demineralized freeze-dried bone allograft (DFDBA) combined with iPRF in treating periodontal intrabony osseous defects, employing cone-beam computed tomography for radiographic evaluation. A total of 28 periodontal intrabony defects were included in this study. Defects assigned to the control arm received DFDBA plus iPRF, while those in the test arm were treated with *P. guajava* leaf powder plus iPRF. Measurements of the gingival index (GI), plaque index (PI), probing pocket depth (PPD), and clinical attachment level (CAL) were taken at baseline, 3 months, and 6 months. In contrast, radiographic assessments occurred at baseline and 6 months. Both study arms achieved meaningful improvements across all clinical and radiographic parameters, with reductions noted in GI, PI, PPD, and CAL at the 3- and 6-month follow-ups. The test arm showed more pronounced CAL gain and more favorable radiographic findings compared to the control arm, though the degree of bone fill proved similar between the two arms. *P. guajava* leaf powder used with iPRF yielded statistically comparable outcomes to DFDBA used with iPRF, suggesting that *P. guajava* leaf powder is a suitable option for periodontal regeneration.

Keywords: Allograft, Cone-beam computed tomography, Injectable platelet-rich fibrin, Intrabony defects, Periodontal regeneration, *Psidium guajava*

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Introduction

Intrabony osseous defects in the periodontium constitute a complex challenge in clinical periodontal care, severely disrupting alveolar bone integrity and accelerating the progression of periodontal disease [1]. Such defects are characterized by bone resorption within the tooth socket, resulting in deepened pockets, attachment loss, and, if left unmanaged, eventual tooth mobility [2]. Proper treatment requires regenerative techniques focused on restoring lost bone and the supporting structures. A variety of novel therapeutic approaches have been studied to optimize clinical outcomes and curb disease advancement [3].

Injectable platelet-rich fibrin (iPRF) has arisen as a valuable adjunct for periodontal regeneration [4]. As a refined form of platelet-rich fibrin (PRF), iPRF is an autologous, natural platelet concentrate obtained by low-speed centrifugation, yielding a liquid that is easy to inject. It supports tissue healing by establishing a scaffold for cell migration and enabling a continuous delivery of growth factors [5, 6]. iPRF has been shown

to accelerate wound repair, alleviate inflammation, and stimulate bone formation, especially when paired with biomaterials such as demineralized freeze-dried bone allograft (DFDBA) [7]. Owing to its osteoinductive capacity, DFDBA promotes new bone formation and has long served as the reference standard for bone regeneration [8].

Recent research has highlighted the potential of plant-derived substances, such as *Psidium guajava* leaf powder (guava leaf powder), to promote tissue regeneration. Rich in bioactive molecules, including flavonoids, tannins, and essential oils, guava leaf powder exerts anti-inflammatory, antimicrobial, and antioxidant actions, making it a strong candidate for periodontal applications [9, 10]. Its promise as a natural, biocompatible grafting material offers a substitute for animal-derived materials such as DFDBA [11]. Moreover, earlier investigations have documented osteoanabolic effects of guava leaf powder, reinforcing its relevance to bone regeneration [12, 13].

This pilot trial aims to evaluate and compare the clinical and radiographic performance of *P. guajava* leaf powder combined with iPRF relative to the well-established pairing of DFDBA with iPRF in the treatment of periodontal intrabony defects, using cone-beam computed tomography (CBCT) to assess bone regrowth precisely.

Materials and Methods

This investigation took the form of a pilot, double-blind, randomized controlled trial encompassing 28 intrabony osseous defects in 10 individuals aged 22–54 years, each with a diagnosis of Stage III Grade A periodontitis. Subjects were drawn from the Department of Periodontics and Implantology. Enrollees were divided between a control arm managed with DFDBA plus iPRF and a test arm managed with *P. guajava* leaf powder plus iPRF **(Figure 1).**

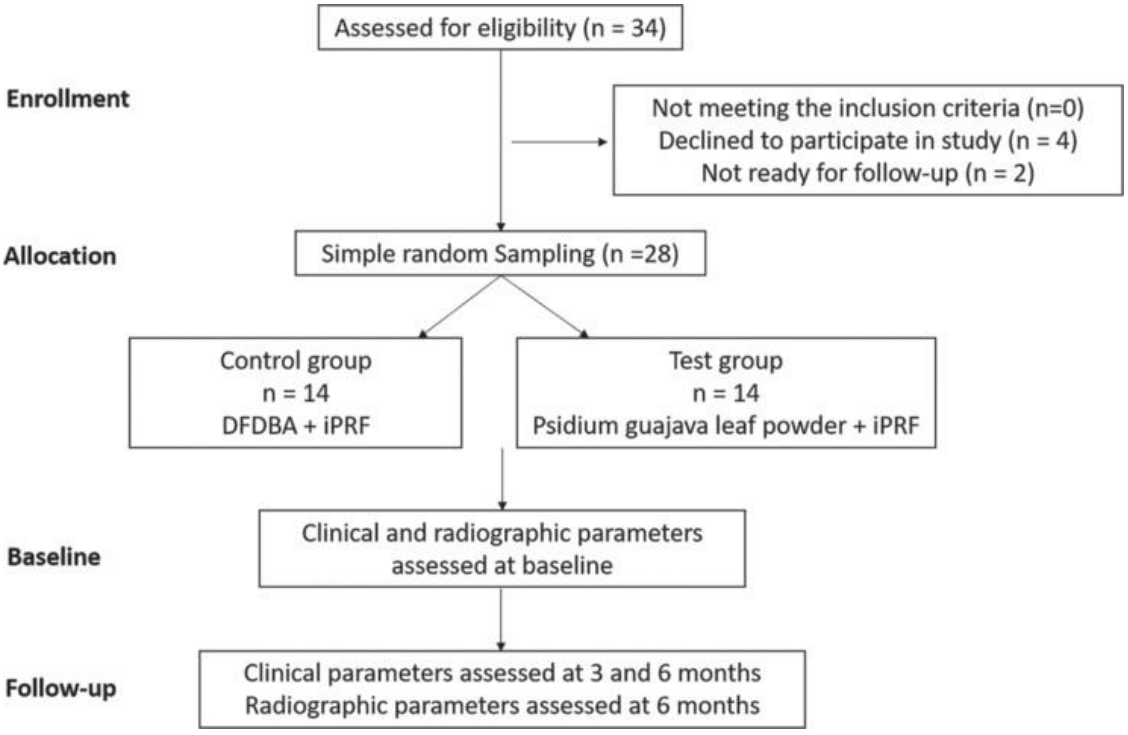


Figure 1. Trial flow diagram

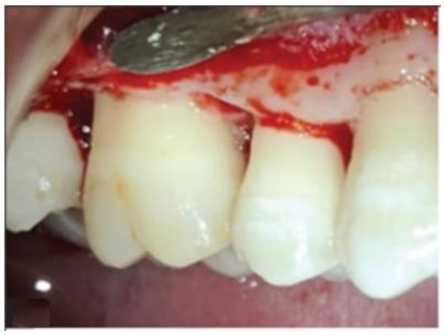
Running from July 2023 to September 2024, the study followed the ethical requirements of the 1975 Declaration of Helsinki, with its 2013 update. The institute's Institutional Committee provided ethical clearance, and the trial was recorded in the Clinical Trials Registry India (CTRI/2023/08/056500).

Participants who held a diagnosis of Stage III Grade A periodontitis, coupled with a PPD of ≥ 6 mm, CAL of ≥ 5 mm, and at least one intraosseous defect involving two or three walls in either jaw, were considered suitable for inclusion. Defects were verified through both clinical and radiographic assessment. The investigation excluded anyone with a background of systemic illness, hypersensitivity, or ongoing medication usage. Subjects who had undergone periodontal care within the past 6 months were similarly disqualified, as were pregnant or lactating females, individuals who smoked or chewed tobacco,

and those with inadequate oral cleanliness (plaque index [PI] > 1.5). Teeth showing mobility exceeding Grade II were also omitted.

Ahead of the surgical phase, every participant completed a presurgical session that included tailored oral hygiene instruction, scaling and root planing, and, when necessary, occlusal adjustment. Plaque control was rechecked 3 weeks after Phase I therapy to guarantee adequacy. Ten patients with 28 intrabony osseous defects were randomly assigned to one of two treatment groups. Group I (control) received DFDBA mixed with iPRF, whereas Group II (test group) received *P. guajava* leaf powder mixed with iPRF. Randomization was executed via a computer-generated number sequence at the time of surgery. Written informed consent was obtained from all individuals before enrollment.

After presurgical therapy concluded, several clinical indices were recorded, among them the gingival index (GI), PI, probing pocket depth (PPD), and clinical attachment level (CAL). These parameters were captured at baseline, 3 months, and 6 months. A periodontal probe was used to measure PPD and CAL. To maintain uniform angulation and positioning across visits, custom acrylic stents with vertically grooved probing reference points were manufactured **(Figure 2).** Radiographic parameters obtained from CBCT scans at baseline and 6 months comprised the following: the height of the intrabony defect, defined as the distance from the cementoenamel junction (CEJ) to the base of the defect (BD) and denoted CEJ-BD, along with the distance from the CEJ to the alveolar crest (AC), denoted CEJ-AC. Defect depth was computed as AC minus BD, designated AC-BD. Further measurements captured the mesiodistal (MD) and buccolingual (BL) extents of the defect and the bone defect volume (BDV). Bone fill was calculated as the difference between baseline BDV and 6-month BDV **(Figure 3)**.



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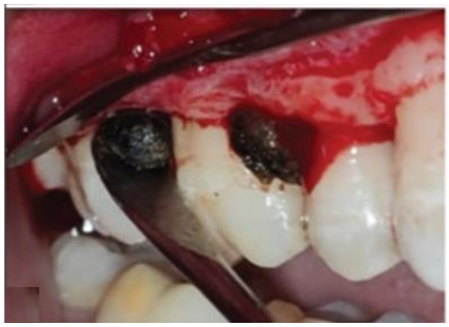

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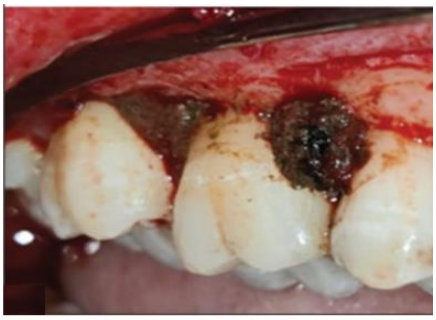

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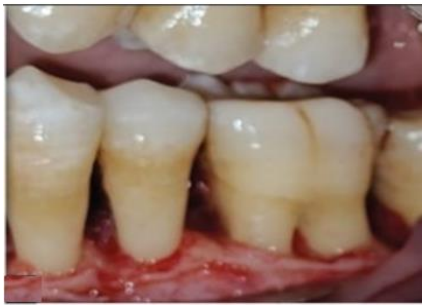

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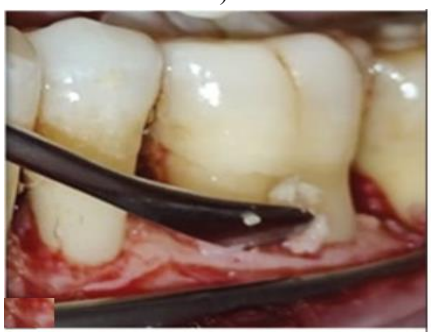

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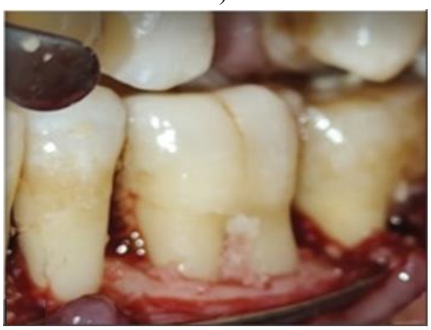

f)

Figure 2. Clinical photographs of the test and control arms: (a–c) Test arm receiving application of *Psidium guajava* leaf powder combined with

iPRF; (d–f) Control arm receiving application of DFDBA combined with iPRF.

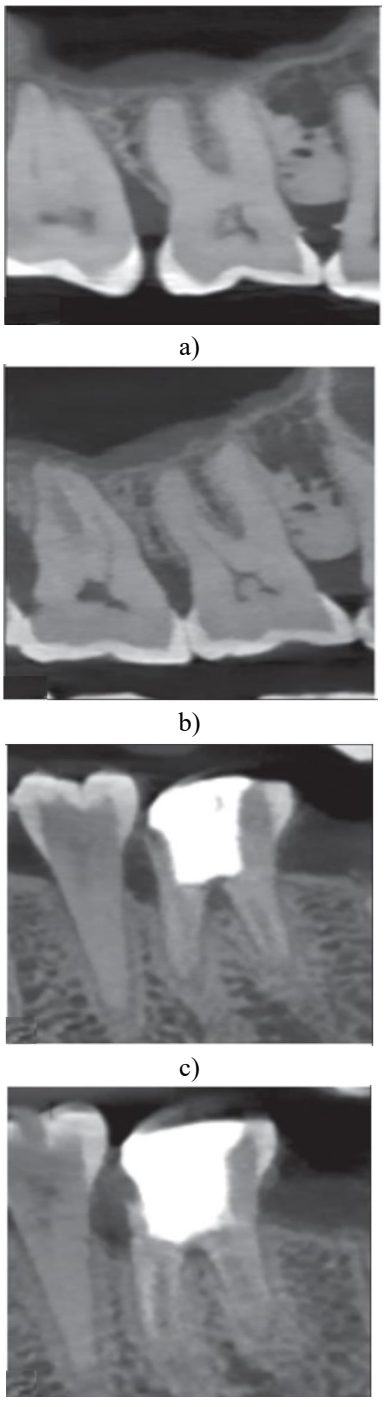

Figure 3. CBCT scans of the test cohort (*Psidium guajava* leaf powder plus iPRF) and the control cohort (DFDBA plus iPRF) were obtained preoperatively and 6 months postoperatively: (a) Preoperative radiolucent area at baseline within the test cohort; (b) Decrease in radiolucent area at the 6-month mark within the test cohort, pointing to bone fill; (c) Preoperative radiolucent area at baseline within the control cohort; (d) Decrease in radiolucent area at the 6-month mark within the control cohort, pointing to bone fill.

For the preparation of iPRF, a venous blood sample was drawn and centrifuged to collect the iPRF layer, which was afterward blended with either DFDBA or *P. guajava* leaf powder, depending on the assigned group. The protocol for producing guava leaf powder was as follows: 10 g of coarsely cut, air-dried guava foliage was mixed with 100 mL of distilled water and processed in a Soxhlet extractor for 6 hours. The mixture was then passed through a filter to eliminate solid particles, and the resulting liquid was concentrated to dryness in a flat-bottomed container set within a thermostatic water bath sustained at 100 °C. Once evaporation was complete, the remaining material was dried to a stable weight, and the yield of the water-soluble extract was calculated as a percentage of the original leaf mass. The resultant extract lends itself to various applications, notably in combination with iPRF for regenerative purposes.

After baseline data gathering and initial therapy, patients were rinsed with a 0.12% chlorhexidine digluconate solution before surgery. A double-blind design was enforced, meaning neither the surgeon nor the outcome assessor knew the group assignments. Local anesthesia was achieved with 2% lidocaine HCl containing adrenaline (1:200,000), and adequate numbness was verified before commencing. An intrasulcular incision was made, and a full-thickness mucoperiosteal flap was raised to expose the underlying bone for thorough furcation debridement. Scaling and root planing were performed as part of the intraoperative protocol. Depending on the group assignment, iPRF blended with DFDBA or *P. guajava* leaf powder was then packed into the intrabony defect. The flaps were repositioned and secured with interrupted sutures, and a periodontal dressing was placed. Postoperative oral hygiene guidance was provided. A 5-day twice-daily regimen of amoxicillin–clavulanic acid and aceclofenac was prescribed. Sutures were removed at 7 days. Patients were counseled to avoid hard or sticky foods and to avoid harsh brushing in the treated zone until the 3-month review. A 0.12% chlorhexidine digluconate mouthrinse (10 mL, twice daily) was used for 15 days following the procedure.

The same acrylic stent was used to record clinical measures at 3 and 6 months, while CBCT imaging was performed 6 months after surgery for radiographic assessment. The 6-month outcome set encompassed PPD, CAL, PI, GI, and bone fill.

Statistical processing was performed with IBM SPSS Statistics for Windows, Version 20.0, introduced in 2011 by IBM Corp., Armonk, New York, USA., adopting $P < 0.05$ as the cutoff for significance. Between-group contrasts for PPD, CAL, and bone fill were conducted using independent t-tests. Within-group variations over time were examined with paired t-tests, and ANOVA served to compare multiple groups across all metrics—PPD, CAL, PI, GI, BDV, and bone fill—at baseline, 3 months, and 6 months.

Results and Discussion

Throughout the study, participants in both groups maintained excellent plaque control and healed uneventfully. A total of 28 intrabony defects were treated. At baseline, 3 months, and 6 months, mean ± standard deviation (SD) GI scores were 1.64 ± 0.35, 1.15 ± 0.36, and 0.77 ± 0.27, while mean ± SD PI scores were 2.18 ± 0.71, 1.30 ± 0.65, and 1.04 ± 0.59, respectively. When the 6-month values were compared to baseline, both groups experienced a statistically significant reduction in GI (highly significant P = 0.000) and PI (significant P = 0.002).

At the outset, probing depths and attachment levels were comparable between the two groups. By the 3-month time point, the control group had realized a marked enhancement in both probing depth and attachment level; the test group likewise progressed, though to a lesser degree. At the 6-month evaluation, both groups continued to show significant gains in PPD and CAL, with progressively better outcomes over time. These improvements reached statistical significance **(Table 1)**.

Table 1. Comparison of probing pocket depth and clinical attachment level across time and between two sites

Time point	Test group (mean ± SD)	Control group (mean ± SD)
PPD (mm)		
Baseline	3.43 ± 0.48	3.21 ± 0.48
3 months	2.16 ± 0.36	1.77 ± 0.33
6 months	1.68 ± 0.41	1.34 ± 0.33
P (CAL)	0.000[b]	0.000[b]
Baseline	3.71 ± 0.68	3.32 ± 0.63
3 months	2.21 ± 0.52	1.77 ± 0.33
6 months	1.80 ± 0.61	1.34 ± 0.33
P-value	0.000[b]	0.000[b]

Statistical significance is denoted by b. Abbreviations: PPD = Probing pocket depth, CAL = Clinical attachment level, SD = Standard deviation

Radiographic outcomes showed pronounced enhancement in both groups from the preoperative time point to the 6-month follow-up. The control group showed reductions in all measured parameters—bone heights and distances between anatomical landmarks. The test group showed corresponding decreases in the same parameters, although the numerical values varied slightly. Overall, noteworthy changes were observed in both groups across all assessed dimensions, with these differences reaching statistical significance **(Table 2)**.

Table 2. Comparison between two time points in each group.

Time point	Test group (mean ± SD)	Control group (mean ± SD)
CEJ–BD (mm)		
Baseline	7.51 ± 1.31	6.79 ± 1.53
6 months	5.49 ± 1.45	4.37 ± 0.92
P-value	0.000[b]	0.000[b]
CEJ–AC (mm)		
Baseline	3.56 ± 0.56	3.34 ± 0.78
6 months	2.94 ± 0.53	2.46 ± 0.43
P-value	0.007[b]	0.007[b]
AC–BD (mm)		
Baseline	3.97 ± 1.36	3.44 ± 1.09
6 months	2.55 ± 1.37	1.84 ± 0.60
P-value	0.000[b]	0.000[b]
MD (mm)		
Baseline	2.73 ± 0.46	3.44 ± 1.09
6 months	2.27 ± 0.41	1.84 ± 0.60
P-value	0.000[b]	0.000[b]
BL (mm)		
Baseline	5.56 ± 1.36	4.93 ± 1.12
6 months	3.90 ± 1.31	3.41 ± 0.86
P-value	0.020[b]	0.004[b]
BDV (mm^3)		
Baseline	58.98 ± 21.45	45.31 ± 25.05
6 months	21.35 ± 12.34	14.19 ± 7.30
P-value	0.000[b]	0.000[b]

Abbreviations: CEJ-BD = Cementoenamel Junction to the base of the defect, CEJ-AC = Cementoenamel Junction to the alveolar crest, AC-BD = Alveolar crest to the base of the defect, MD = Mesiodistal dimension, BL = Buccolingual dimension, and BDV = Bone defect volume

Initially, PPD and CAL did not differ significantly between the two arms. By the 3-month evaluation, the test arm showed a notably higher PPD, and this difference persisted at 6 months, indicating a sharper decrease in PPD in the control arm. Conversely, the test arm achieved more pronounced CAL gains at both the 3-month and 6-month assessments. Radiographic

parameters displayed no disparities at baseline. At the 6-month mark, the test arm demonstrated markedly greater bone dimensions in selected regions, whereas other areas remained comparable. Bone fill values were similar across the two arms, with no statistically significant difference observed **(Table 3)**.

Table 3. Comparison between the two groups at each time point.

Parameter/ Time point	P-value	Test group (mean ± SD)	Control group (mean ± SD)
PPD (mm)			
Baseline	0.250[c]	3.43 ± 0.48	3.21 ± 0.48
3 months	0.006[b]	2.16 ± 0.36	1.77 ± 0.33
6 months	0.024[b]	1.68 ± 0.41	1.34 ± 0.33
CAL (mm)			
Baseline	0.125[c]	3.71 ± 0.68	3.32 ± 0.63
3 months	0.013[b]	2.21 ± 0.52	1.77 ± 0.33
6 months	0.019[b]	1.80 ± 0.61	1.34 ± 0.33
CEJ–BD (mm)			
Baseline	0.187[c]	7.51 ± 1.31	6.79 ± 1.53
6 months	0.022[b]	5.49 ± 1.45	4.37 ± 0.92
CEJ–AC (mm)			
Baseline	0.412[c]	3.56 ± 0.56	3.34 ± 0.78
6 months	0.012[b]	2.94 ± 0.53	2.46 ± 0.43
AC–BC (mm)			
Baseline	0.272[c]	3.97 ± 1.36	3.44 ± 1.09
6 months	0.089[c]	2.55 ± 1.37	1.84 ± 0.60
MD (mm)			
Baseline	0.622[c]	2.73 ± 0.46	2.62 ± 0.66
6 months	0.661[c]	2.27 ± 0.41	2.19 ± 0.51
BL (mm)			
Baseline	0.188[c]	5.56 ± 1.36	4.93 ± 1.12
6 months	0.257[c]	3.90 ± 1.31	3.41 ± 0.86
BDV (mm³)			
Baseline	0.133[c]	58.98 ± 21.45	45.31 ± 25.05
6 months	0.073[c]	21.35 ± 12.34	14.19 ± 7.30
Bone fill (mm³)			
—	0.404[c]	37.62 ± 19.34	31.12 ± 21.19

[b]: Significant, [c]: Not significant. Abbreviations: PPD = Probing pocket depth, CAL = Clinical attachment level, CEJ-BD = Cementoenamel junction to the base of the defect, CEJ-AC = Cementoenamel junction to the alveolar crest, AC-BC = Alveolar crest to the base of the defect, MD = Mesiodistal dimension, BL = Buccolingual dimension, BDV = Bone defect volume, SD = Standard deviation.

Periodontal disorders, periodontitis chief among them, stretch across populations worldwide and regularly trigger alveolar bone erosion. Intrabony defects, typified by vertical osseous destruction, are classified as 1-, 2-, or 3-wall defects. The present work concentrated on 2- to 3-wall intrabony defects, as such configurations offer a truer window into the regenerative capacity attainable under clinical conditions.

Turning to plant-derived preparations for periodontal regrowth has attracted growing interest owing to their anti-inflammatory, antimicrobial, and osteoinductive properties. Sabu *et al*. [14] reported that Morinda citrifolia fruit extract increased bone fill by 27.7% in periodontal lesions, as assessed by CBCT, further supporting the notion that botanical concentrates may serve as substitutes for conventional modalities.

One such botanical entrant, *P. guajava*—widely known as guava leaf powder—has lately come under the spotlight for its expansive therapeutic repertoire. Multiple reports have spotlighted its conceivable contribution to periodontal healing. Porwal *et al*. [12] assessed the osteogenic potency of *P. guajava* fruit extract and demonstrated that its triterpene constituents exhibit osteoanabolic activity. Their data revealed a robust osteogenic response to *P. guajava* fruit extract, stimulating bone formation and suggesting its utility in bone-restorative regimens [12]. Separately, Tanideh *et al*. [13] investigated the effects of *P. guajava* leaf essential oil on osteoarthritis in an animal model and found that it promoted bone repair, thereby validating its relevance for skeletal disorders.

Sharma *et al*. [15] investigated the adjunctive role of a 3% *P. guajava* gel formulation in chronic periodontitis therapy in a split-mouth trial. Their findings documented substantial improvements in clinical indices such as PPD and CAL, as well as a decline in colony-forming units of *A. actinomycetemcomitans* and *P. gingivalis* within test sites at the 1- and 3-month time points. These outcomes imply that *P. guajava* gel could serve as an effective supplementary agent in the care of chronic periodontitis [15].

Within the current investigation, *P. guajava* leaf powder was coupled with iPRF to address periodontal intrabony defects. iPRF, a fluid variant of PRF, has been established as a supportive agent for periodontal regeneration. Ghoderao *et al*. [7] demonstrated that iPRF, when blended with bone graft materials, yields superior clinical outcomes, including greater bone fill and attachment gain. In a similar vein, Dindarini *et al*. [16] noted that iPRF combined with bone grafts markedly improved clinical variables, such as PPD and CAL. These prior observations accord well with the data emerging from the present study. The present work also demonstrated that *P. guajava* leaf powder combined with iPRF yielded results comparable to those obtained with DFDBA, widely regarded as the

reference standard for osseous regeneration in periodontal defects. Preceding investigations, including one by Samarth *et al*. [17], have shown that pairing DFDBA with a CGF membrane results in noticeable clinical and radiographic improvements, affirming its role in periodontal regenerative therapy.

On the clinical front, this trial registered a drop in PPD and a gain in CAL within both arms. Radiographic scrutiny demonstrated significant progress across all parameters, with the two arms exhibiting comparable changes in CEJ-BD, CEJ-AC, and AC-BD. However, the test arm outperformed in CEJ-BD and CEJ-AC. The deployment of CBCT enabled a meticulous and trustworthy evaluation of bone regrowth, consistent with prior studies that emphasize CBCT's diagnostic merits [18]. These data corroborate that *P. guajava* leaf powder in conjunction with iPRF represents a credible alternative to DFDBA for the regeneration of periodontal intrabony defects. The incorporation of maxillary alongside mandibular dentition as both test and control sites may engender some degree of variability arising from inherent differences in bone porosity, circulatory supply, and anatomic configuration—a limitation duly noted. Moreover, the restricted participant pool and the lack of histological assessment narrow the interpretative reach of this work, underscoring the need for future studies with larger samples and longer follow-up intervals.

Conclusion

Within the confines of this pilot investigation, one may deduce that *P. guajava* leaf powder—owing to its advantageous attributes—has potential as a bone-filling, regenerative material when employed alongside iPRF. The outcomes point to effects on a par with those of the benchmark DFDBA, a conclusion reinforced by CBCT imaging.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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