

# Assessment of Anti-Protease Property of Nutmeg in Causing Delayed Disintegration of Platelet Rich Fibrin – an *in vitro* Study

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## ABSTRACT

**Background:** Platelet-rich fibrin is a second generation platelet concentrate enhances tissue healing and is in predominant use as a barrier membrane in periodontal regeneration. However, a normal PRF membrane has rapid degradability (1-2 weeks). *Myristica fragrans* (nutmeg) has been found to have antiprotease property. It was hypothesized if this property helps in inhibiting degradation of PRF. **Aim:** To assess whether nutmeg has any effect in inhibiting degradability of PRF membrane and to compare the degradability of PRF at different concentrations (200mg, 100mg, 50mg) of ethanolic and crude extracts of nutmeg. **Materials and Methodology:** PRF was procured from 30 ml blood from 5 different donors were cut to equal sizes into 35 pieces. They were measured at baseline and dropped in 7 sets of ependorfs containing PBS, PBS containing 200 mg, 100mg and 50 mg crude extract of nutmeg, PBS containing 200 mg, 100mg and 50 mg ethanolic extract of nutmeg. After 1 week the PRF were retrieved and measured. The percentage of remaining PRF was calculated and data analysed. **Result:** It was found that there was a difference in percentages of remaining PRF between all the groups when compared to the control group, out of which, crude extract of nutmeg 200 mg group alone had a significantly lesser % of remaining PRF than the control. All ethanolic extract groups had a significantly greater % of remaining PRF when compared to that of the control. **Conclusion:** Nutmeg is effective in inhibiting the degradation of PRF membrane.

**Key words:** Platelet-rich fibrin, *Myristica fragrans*, Proteolysis, Periodontal guided tissue regeneration, Periodontitis.

## INTRODUCTION

Periodontal disease is an inflammatory disease characterized by the destruction of periodontal tissues. The aim of periodontal therapy is to regulate inflammatory process, prevent the progression of periodontal disease and to regenerate the lost periodontal tissues. Periodontal regeneration is a multifactorial process involving cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence.<sup>1</sup> There are various regenerative therapies available including soft tissue grafts, hard tissue grafts, guided tissue regeneration, root bio modification or a combination of these.<sup>2</sup> However, regenerative periodontal therapies have do not attain complete periodontal restoration.<sup>2,3</sup> Various biomaterials have been used in periodontal tissue regeneration in addition to bone grafts.

Periodontal wound healing includes a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells and osteoblasts. The disruption of vasculature during wound healing leads to fibrin formation, platelet aggregation, and release of several growth factors<sup>4</sup> into tissues from platelets through molecular signals which are primarily mediated by cytokines and growth factors. The presence of growth factors and cytokines in platelets are important

in inflammation and wound healing.<sup>5</sup> Platelets also secrete fibrin, fibronectin, and vitronectin, which act as a matrix for the connective tissue and as adhesion molecules for more efficient cell migration. Thus, it is wise to use platelets as therapeutic tools to improve tissue repair.

Platelet-rich fibrin (PRF) is a platelet concentrate enhances tissue healing. It was first introduced by Choukroun *et al.*<sup>6</sup> with simplified processing and without biochemical blood handling. The use of platelet gel to improve bone regeneration is a recent technique in implantology. However, the biologic properties and real effects of such products remain controversial. It is advantageous over platelet-rich plasma due to its ease of preparation, ease of application and minimal expense.

Another added advantage of PRF is the presence of natural fibrin network in PRF which protects the growth factors from proteolysis. PRF also favours the development of micro vascularization leading to a more efficient cell migration.

J Mironet *al.*<sup>7</sup> in his review article has concluded that the use of PRF has gained tremendous momentum

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as a low cost biological scaffold capable of improving tissue healing. Patil *et al.*<sup>8</sup> has presented a case report, reporting the successful treatment of radicular cysts using autologous periosteum and platelet-rich fibrin with demineralized freeze-dried bone allograft. Panda *et al.*<sup>9</sup> in his randomised controlled trial has concluded that the adjunctive use of PRF in combination with barrier membrane is more effective in the treatment of intrabony defects in chronic periodontitis as compared with barrier membrane alone.

Hafez *et al.*<sup>10</sup> in his case series study concluded that Platelet rich fibrin as a membrane provided good soft tissue coverage over the immediate implants and it enhanced bone stability. The technique was easy to perform with good esthetic results. Fernando *et al.* concluded in his case report that the PRF membranes were excellent alternatives to all alloplastic membranes, providing the necessary protection for bone formation. Ahmed *et al.* in his clinical trial has concluded that addition of PRF to deprotonized bovine bone might be a useful adjunct for alveolar ridge augmentation around immediate implant placement.

Hartshorne *et al.*<sup>11</sup> in his review had concluded that the benefits derived from using PRF in various clinical applications for promoting wound healing and tissue regeneration, its antibacterial and anti-haemorrhagic effects, the low risks with its use, and the availability of easy and low cost preparation methods, should encourage more clinicians to adopt this technology in their practice for the benefit their patients. Aroca *et al.*<sup>12</sup> in her clinical trial where PRF was added to modified coronally advanced flap for treatment of adjacent multiple recession coverage procedures, concluded that the addition of a PRF membrane positioned under the modified coronally advanced flap (MCAF) provided inferior root coverage but an additional gain in gingival/mucosal thickness (GTH) at 6 months compared to conventional therapy.

An *in vitro* study by Sam *et al.*<sup>13</sup> have proven that PRF has a higher degradability and less rigidity when compared to commercially available barrier membranes. This causes PRF to get resorbed faster and get collapsed thus causing failure in space maintenance in guided tissue regeneration.

Isobe *et al.*<sup>14</sup> in an *in vitro* study compared various mechanical and chemical properties of PRF and concentrated growth factor (CGF), including degradability, which was tested by a digestive test using Phosphate buffered saline (PBS) solution containing trypsin and Ethelenediaminetetraacetic acid (EDTA) and concluded that both had similar mechanical and chemical properties.

Yamashita *et al.*<sup>15</sup> in their *in vivo* histological study in rats studied the stability of PRF, and concluded that the histologically and immunohistochemically, the PRF became small and sparse at 21 days, almost disappeared at 28 days. Hence, although PRF can potentially stimulate tissue regeneration, its degradation time should be considered in its clinical applications. It is also obvious that PRF alone cannot work as a scaffold or a barrier membrane for maintaining the space of regeneration.

Myristica fragrans also known as 'nutmeg' is popular in most parts of West Africa as a spice. However, the essential oil of nutmeg is used externally for rheumatism and internally as a carminative. It is also used in soups as a postpartum medication. Compounds isolated from the seeds of this plant have been reported to possess strong platelet anti-aggregatory activity.<sup>16</sup>

In a study by Lee KK *et al.*<sup>17</sup> the inhibitory effects of 150 medicinal plants on elastase activity were investigated. Among the 150 plants, myristica fragrans (nutmeg) and 5 other plant extracts exhibited more than 65% of inhibition of elastase activity. In the present study, we hypothesized that myristica fragrans (nutmeg) may prolong the degradation of PRF.

Thus this *in vitro* study aimed to assess whether nutmeg has any effect in inhibiting degradability of PRF membrane and to compare the

degradability of PRF at different concentrations (200 mg, 100 mg, 50 mg) of ethanolic and crude extracts of nutmeg.

## MATERIALS AND METHODS

The study was conducted in the dental research lab in saveetha institute of medical and technological sciences. In this study, the degradability of PRF membrane was compared between crude and ethanolic extracts of nutmeg at various concentrations such as 50mg, 100mg, and 200mg against negative control of Phosphate buffer solution(PBS).

The different groups in this study are: Group 1- PBS (negative control) N=5, Group 2- PBS containing crude extract of nutmeg 200 mg N=5, Group 3- PBS containing crude extract of nutmeg 100 mg N=5, Group 4- PBS containing crude extract of nutmeg 50 mg N=5, Group 5- PBS containing ethanolic extract of nutmeg 200 mg N=5, Group 6- PBS containing ethanolic extract of nutmeg 100 mg N=5 and Group 7- PBS containing ethanolic extract of nutmeg 50 mg N=5.

Commercially available finely grounded nutmeg powder was used for preparation of crude and ethanolic extracts of nutmeg. For the preparation of ethanolic extract of nutmeg, 5 mg nutmeg was dropped in 20 ml ethanol. It could evaporate over a period of 5 days. The resulting extract was collected. 50, 100 and 200mg of these extracts were measured in a micro weighing scale and mixed in 1ml PBS in separate eppendorfs. For the preparation of crude extract of nutmeg, 50, 100 and 200mg was measured in a micro weighing scale and mixed in 1 ml PBS in separate eppendorfs.

The blood donors for PRF procurement selected were systemically healthy student volunteers. 30 ml venous blood from 5 different donors were collected. The preparation of PRF was done based on the procedure described by Choukran *et al.*<sup>6</sup> with simplified processing and without biochemical blood handling. The blood collected was dispensed in 30 test tubes. It was run on a centrifuge at 2500 rates per minute for 15 min in small batches after which it settles into the following layers: red lower fraction containing red blood cells, upper straw coloured cellular plasma and the middle fraction containing the fibrin clot: The upper straw coloured layer was then removed and middle fraction was collected, 2 mm below lower dividing line, which is the PRF. PRF procured from each donor was pressed between sterile gauze pieces. They were cut into equal sizes of 7×3 mm. They were thoroughly dried in a blotting paper and excess water was removed to eliminate errors in weight measurement. The pieces were weighed in a micro weighing machine (REMI 1MLH). They were later dropped in each of the 7 groups of eppendorfs (5 each in group). After 1 week of storing in room temperature, the PRF pieces were retrieved, dried in a blotting paper and weighed again. Percentage of remaining PRF [(weight at 1 week / weight at baseline) × 100] was calculated in each Eppendorf. The mean value of all the percentage was calculated in each group. The data was analysed using SPSS software (Statistical Presentation System Software, 1999, SPSS Inc., New York, version 10.0). ANOVA and post hoc tests were used to statistically compare this data between the groups.

## RESULTS

The mean weight of PRF at baseline in the groups 1(PBS) Group 2(PBS + 200 mg crude extract of nutmeg), group 3 (PBS + 100 mg crude extract of nutmeg), Group 4 (PBS + 50 mg crude extract of nutmeg), Group 5 (PBS + 200 mg ethanolic extract of nutmeg), Group 6 (PBS + 100 mg ethanolic extract of nutmeg), Group 7 (PBS + 50 mg ethanolic extract of nutmeg) were 14.6±2.83, 19.74±5.12, 18.36±2.23, 18±4.15, 18.88±2.83, 18.62±4.51, 19.37±3.71 respectively. The mean weight of PRF at 1 week of groups 1-7 were 5.18±0.85, 2.38±2.6, 4.54±3.3, 5.76±1.81, 16.04±2.2, 15.16±4.82, 17.26±3.04 respectively. There was a reduction in the

weights of the PRF in all the groups at 1 week when compared to baseline. The mean percentage of remaining weight of PRF in groups 1-7 were 35.59±14.23, 10.7±11.92, 23.71±17.85, 85.09±2.89, 34.86±17.65, 85.09±2.89, 79.81±9.02, 86.01±4.42 respectively. (Table 1)

The percentage of remaining weight of PRF at 1 week was maximum in Group 7 (PBS + 50 mg ethanolic extract of nutmeg), followed by Group 5 (PBS + 200 mg ethanolic extract of nutmeg), Group 6 (PBS + 100 mg ethanolic extract of nutmeg), Group 1 (PBS - Control), Group 4 (PBS + 50 mg crude extract of nutmeg), Group 3 (PBS + 100 mg crude extract of nutmeg) and the least in Group 2 (PBS + 200 mg crude extract of nutmeg). (Table 1)

When the remaining weight of PRF in 7 groups were compared statistically using ANOVA, the difference was statistically significant with a P value of 0.00. (Table 2)

On Post hoc comparison, when each of the test groups (Group 2 – 7) were compared against the control group (Group 1) individually, the difference was statistically significant only in the group 2, group 5, group 6 and group 7. On analysing the result, it was found that, all the three concentrations of ethanolic extract were significantly better than control group, whereas group 2 (PBS + 200mg of crude extract of nutmeg) was found to be significantly worse than the control group. However, Group 3 (PBS + 100mg crude extract of nutmeg) and 4 (PBS + 50mg crude extract of nutmeg), did not have any significant difference with the control group. (Table 3)

## DISCUSSION

The present study was aimed to assess whether nutmeg has any effect in inhibiting degradability of PRF membrane and to compare the degrad-

ability of PRF at different concentrations (200 mg, 100 mg, and 50 mg) of ethanolic and crude extracts of nutmeg.

The rationale behind the use of PRF membrane is that the platelet α granules have many Growth factors (GF)s including Platelet derived GFs, Transforming GF-β, Vascular endothelial GF, and Epidermal GF.<sup>18</sup> PRF has to be considered as a fibrin biomaterial that are important in hard and soft tissue repair mechanism. Its molecular structure is an optimal matrix for migration of endothelial cells and fibroblasts. It permits angiogenesis and remodelling of fibrin in a more resistant connective tissue.<sup>6</sup> With simplified processing and without biochemical blood handling.

Choukroun *et al.*<sup>6</sup> introduced the usage of PRF and came up with a protocol for its preparation for use in oral and maxillofacial surgery to improve bone healing in implant dentistry. Many studies<sup>19-23</sup> have established the clinical and radiographic efficiency of PRF in periodontal defects. The addition of PRF as a membrane to coronally advanced flap showed an increase in width of keratinized gingiva.<sup>12,24</sup> Thus, it is wise to extend the use of PRF membrane to guided tissue regeneration (GTR) applications as well. The assessment of the mechanical properties of PRF membrane for GTR procedures is required before its clinical use.

But, the structural integrity of the implanted bio absorbable barrier membrane should be preserved for a sufficient time to ensure desired results. In the study by Sam *et al.*<sup>13</sup> the degradation rate of PRF *in vitro* was compared with that of two other commercially available collagen membranes to obtain a gross assessment of its degradation profile. The membrane degradation test results showed that PRF membrane was comparable to other membranes in terms of maintaining its physical property up to 6 days. At the end of 1-week, PRF membrane was found to have degraded to about 36% of initial weight, whereas fish collagen to

**Table 1: Mean weight at baseline and 1 week in all the groups.**

	Control Group 1	Crude extract			Ethanolic extract		
		200 mg Group 2	100 mg Group 3	50 mg Group 4	200 mg Group 2	100 mg Group 3	50 mg Group 7
Mean weight at baseline	14.6±2.83	19.74±5.12	18.36±2.23	18±4.15	18.88±2.83	18.62±4.51	19.37±3.71
Mean weight at 1 week	5.18±0.85	2.38±2.6	4.54±3.3	5.76±1.81	16.04±2.2	15.16±4.82	17.26±3.04
% of remaining PRF(M± SD)	35.59±14.23	10.7±11.92	23.71±17.85	34.86±17.65	85.09±2.89	79.81±9.02	86.01±4.42

**Table 2: Comparison of percentage of remaining PRF between the groups by ANOVA.**

	Control Group 1	Crude extract			Ethanolic extract			P
		200 mg Group 2	100 mg Group 3	50 mg Group 4	200 mg Group 5	100 mg Group 6	50 mg Group 7	
% of remaining PRF(M± SD)	35.59±14.23	10.7±11.92	23.71±17.85	34.86±17.65	85.09±2.89	79.81±9.02	86.01±4.42	0.00*

\*significant at P < 0.05

**Table 3: Comparison of percentage of remaining PRF between the groups by post hoc.**

Variable I	Variable J	Mean Difference (I-J)	Std. Error	Sig.
Group 1	Group 2	26.00400	7.50078	.025*
	Group 3	12.98600	7.50078	.602
	Group 4	1.84000	7.50078	1.000
	Group 5	-48.39000	7.50078	.000*
	Group 6	-43.10800	7.50078	.000*
	Group 7	-49.31200	7.50078	.000*

\*significant at P < 0.05



about 8%, and bovine collagen to about 3%. Hence, in the present study, we were looking to find a way to prolong the degradation rate of the PRF. In an *in vitro* study<sup>17</sup> comparing 150 medicinal plants, nutmeg and 5 other plant extracts was found to have inhibitory activity on porcine pancreatic elastase. Thus, in the present study, we hypothesized that addition of nutmeg may have an inhibitory activity on PRF degradation. There was a reduction in the weights of the PRF in all the groups at 1 week when compared to baseline. There was least reduction in Group 7, closely followed by Group 5, Group 6, Group 1, Group 4, Group 3 and Group 2 showing the highest reduction. The reduction is due to the physical property of autogenous PRF that leads to resorption. Yamashita *et al.*<sup>15</sup> has shown that PRF completely disappeared in 28 days in Wistar rat models *in vivo*. The study by Sam *et al.*<sup>13</sup> reports degradation in *in vitro* condition too. Hence, it is proved that PRF degrades in both *in vitro* and *in vivo* conditions.

There was a greater percentage of remaining PRF in the ethanolic extracts when compared to control. The ethanolic extract of nutmeg in all concentrations (200 mg, 100 mg, and 50 mg) showed a greater remaining PRF than the control with the highest being in 50mg ethanolic extract of nutmeg followed by 200 mg and 100 mg. This maybe attributed to the antiprotease effects enumerated in Lee *et al.*<sup>17</sup> and Jangid *et al.*<sup>16</sup>

There was a lesser percentage of remaining PRF in the crude extract when compared to control. The crude extract of nutmeg in all concentrations (200 mg, 100 mg, 50 mg) showed a lesser remaining PRF than the control with the highest being in 50 mg crude extract of nutmeg followed by 100mg and 200 mg. A study by Bae *et al.*<sup>25</sup> showed equal antioxidase property in both the water and ethanolic extracts Rumex Acitosa. Whereas a study by Singh *et al.*<sup>26</sup> showed greater antioxidase effect in crude extracts than ethanolic extracts. This may be attributed to the different solubility properties of different plants. In the present study, since nutmeg is insoluble in water, that may be a reason for its inadequate inhibitory effect on PRF degradation when compared to ethanolic extract.

The uniqueness of this study lies in the fact that a plant extract is being used to inhibit the degradation of PRF thus enhancing its property as a GTR membrane. This study is the first of its kind. However, this is an *in vitro* study. Thus, the property of the PRF and the effect of nutmeg on it may change in an *in vivo* condition. Also, in this *in vitro* study, it was not tested if nutmeg has any influence in the growth factor releasing property of PRF. Further cell culture studies, animal and human histological studies are needed to check the same before establishing it as a drug of therapeutic use in inhibiting PRF degradation, thereby enhancing the clinical efficiency of PRF in periodontal regeneration.

## CONCLUSION

The ethonolic extract of nutmeg is effective in inhibiting the degradation of PRF membrane thus prolonging its function as a barrier membrane and providing space maintenance for a longer time.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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## ABBREVIATIONS

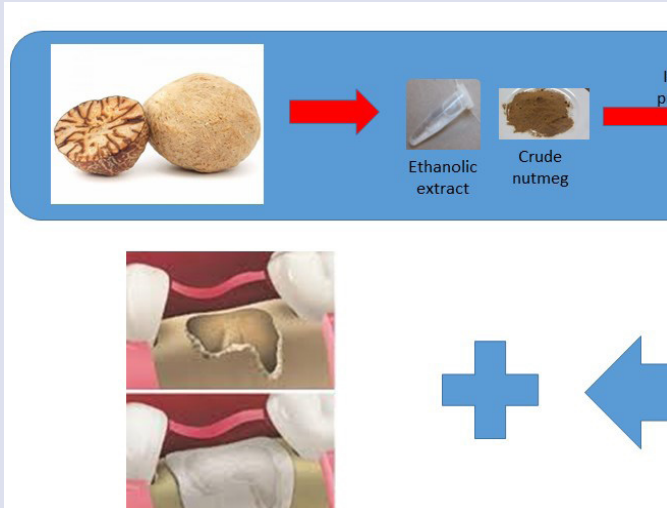
**PRF:** Platelet-rich fibrin; **MCAF:** Modified coronally advanced flap; **GTH:** Gingival/mucosal thickness; **CGF:** Concentrated growth factor; **PBS:** Phosphate buffered saline; **EDTA:** Ethelenediaminetetraacetic acid; **GF:** Growth factor; **GTR:** Guided tissue regeneration;

## REFERENCES

1. The potential role of growth and differentiation factors in periodontal regeneration. J Periodontol. 1996;67:545-53.
2. Greenwell H, Committee on Research, Scienc, Therapy. American Academy of Periodontology. Position paper: Guidelines for periodontal therapy. J Periodontol. 2001;72(11):1624-8.
3. Sander L, Karring T. Healing of periodontal lesions in monkeys following the guided tissue regeneration procedure a histological study. J Clin Periodontol. 1995;22(4):332-7.
4. Deodhar AK, Rana RE. Surgical physiology of wound healing: a review. J Postgrad Med. 1997;43(2):52-6.
5. Giannobile WV. Periodontal tissue engineering by growth factors. Bone. 1996;19(1):23S-37S.
6. Choukroun J, *et al.* Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg. Oral Med. Oral Pathol Oral Radiol. Endod. 2009;101(3):299-303.
7. Miron R, Pikos M. PRF as a Barrier Membrane in Guided Bone Regeneration. Dent Today. 2017;36.
8. Patil V, *et al.* A Novel Approach for Treatment of an Unusual Presentation of Radicular Cysts Using Autologous Periosteum and Platelet-Rich Fibrin in Combination with Demineralized Freeze-Dried Bone Allograft. Case Rep. Dent. 2013;893791.
9. Panda S, *et al.* Adjunctive Effect of Autologous Platelet-Rich Fibrin to Barrier Membrane in the Treatment of Periodontal Intra-bony Defects. J Craniofac Surg. 2016;27(3):691-6.
10. Hafez W K, Seif S A, Shawky H, Hakam M M. Platelet rich fibrin as a membrane for coverage of immediate implants: Case-series study on eight patients. Tanta Dent J. 2015;12(3):203-10.
11. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part III: Clinical indications of PRF in implant dentistry, periodontology, oral surgery and regenerative endodontics - Semantic Scholar. Available at: <https://www.semanticscholar.org/paper/A-comprehensive-clinical-review-of-Platelet-Rich-F-Hartshorne/9a7071ae14fd1c103b6c0cbfa2654cf1fe1f094>. (Accessed: 1st March 2018)
12. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: A 6-month study. J Periodontol. 2009;80(2):244-52.
13. Sam G, Vadakkukkuttal RJ, Amol NV. *In vitro* evaluation of mechanical properties of platelet-rich fibrin membrane and scanning electron microscopic examination of its surface characteristics. J Indian Soc Periodontol. 2015;19(1):32.
14. Isobe K, *et al.* Mechanical and degradation properties of advanced platelet-rich fibrin (A-PRF), concentrated growth factors (CGF), and platelet-poor plasma-derived fibrin (PPTF). Int J Implant Dent. 2017;3(1):17.
15. Yamashita Y, Chen K, Kuroda S, Kasugai S. Stability of Platelet-rich Fibrin *in vivo*: Histological Study in Rats. J Oral Tissue Eng. 2016;14(2):83-90.
16. Jangid K, Jayakumar N, Varghese S. Achievable therapeutic effects of myristica fragrans (NUTMEG) on periodontitis a short review. Int J Pharm Pharm Sci. 2014;6:591-4.
17. Lee KK, Kim JH, Cho JJ, Choi JD. Inhibitory Effects of 150 Plant Extracts on Elastase Activity, and Their Anti-inflammatory Effects. Int J Cosmet Sci. 1999;21(2):71-82.
18. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. *In vitro* release of growth factors from platelet-rich fibrin (PRF): A proposal to optimize the clinical applications of PRF. Oral Surg. Oral Med. Oral Pathol. Oral Radiol Endod. 2009;108(1):56-61.
19. Lekovic V, *et al.* Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intra-bony periodontal defects. J Periodontol Res. 2012;47(4):409-17.
20. Sharma A, Pradeep AR. Treatment of 3-wall intra-bony defects in patients with chronic periodontitis with autologous platelet-rich fibrin: A randomized controlled clinical trial. J Periodontol. 2011;82(12):1705-12.
21. Thorat M, Pradeep AR, Pallavi B. Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: A controlled clinical trial. J Clin Periodontol. 2011;38(10):925-32.
22. Joseph VR, Raghunath A, Sharma N. Clinical effectiveness of autologous platelet rich fibrin in the management of intra-bony periodontal defects. Singapore Dent J. 2012;33(1):5-12.

23. Sharma A, Pradeep AR. Autologous platelet-rich fibrin in the treatment of mandibular degree II furcation defects: A randomized clinical trial. *J Periodontol.* 2011;82(10):1396-403.
24. Padma R, *et al.* A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects. *J Indian Soc Periodontol.* 2013;17(5):631-6.
25. Bae JY, *et al.* A Comparison between Water and Ethanol Extracts of *Rumex acetosa* for Protective Effects on Gastric Ulcers in Mice. *Biomol Ther.* 2012;20(4):425-30.
26. Singh N. A comparison of both water and ethanol extracts prepared from *Echinacea purpurea* and *Echinacea angustifolia* on the response to Influenza A/PR/8/34 infection in mice. *Grad Theses Diss.* 2010.

### GRAPHICAL ABSTRACT



### SUMMARY

- The aim of this study was to assess whether nutmeg has any effect in inhibiting degradability of PRF membrane and to compare the degradability of PRF at different concentrations (200 mg, 100 mg, and 50 mg) of ethanolic and crude extracts of nutmeg. PRF was procured from 30 ml blood from 5 different donors were cut to equal sizes into 35 pieces. They were measured at baseline and dropped in 7 sets of ependorfs containing PBS, PBS containing 200 mg, 100mg and 50 mg crude extract of nutmeg, PBS containing 200 mg, 100mg and 50 mg ethanolic extract of nutmeg. After 1 week the PRF were retrieved and measured. The percentage of remaining PRF was calculated and data analysed. It was found that there was a difference in percentages of remaining PRF between all the groups when compared to the control group, out of which, crude extract of nutmeg 200 mg group alone had a significantly lesser % of remaining PRF than the control. All ethanolic extract groups had a significantly greater % of remaining PRF when compared to that of the control. Hence, it was found that nutmeg is effective in inhibiting the degradation of PRF membrane.

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