

A CLINICAL AND MICROBIOLOGICAL STUDY TO ASSESS THE EFFICACY OF *ACMELLA OLERACEA* AND *ACACIA CATECHU* HERBS AS LOCAL DRUG DELIVERY IN TREATMENT OF CHRONIC GENERALIZED PERIODONTITIS PATIENTS

Jokkin Jesima¹, Harish Saluja^{1*}, Hafees Rahman¹, Pawan Karwa¹

¹ Microbiology Laboratory, Department of Botany, Berhampur University, Bhanja Bihār, Berhampur, Odisha, 760007, India. Harishsaluja41@yahoo.com

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ABSTRACT

A randomized controlled clinical trial was conducted to evaluate the effect of local drug delivery (LDD) of *Acmella oleracea* (*Akarkara*) and *Acacia catechu* (*kattha*) gels as an adjunct to scaling and root planing (SRP) in generalized chronic periodontitis patients. Thirty patients with ninety sites aged 25–65 years with generalized chronic periodontitis having pocket probing depth (PPD) of more than or equal to 5 mm on the basis of inclusion and exclusion criteria were selected. They were treated according to split-mouth design protocol. The placement of gel containing *A. oleracea* (1%) (Group 1) and *A. catechu* (1%) (Group 2) as LDD was placed at the two test sites, respectively, and only SRP was done in the control group (Group 3). Periodontal dressing was applied to all the three sites after procedure, and patients were given oral care guidelines. Evaluation of clinical parameters gingival index (GI), plaque index (PI), gingival bleeding index (GBI), PPD, clinical attachment level (CAL), and relative attachment level (RAL) was done at baseline, 1, 3, and 6 months, and microbiological parameter was assessed at baseline and 6 months. N-benzoyl-DL-arginine-2-naphthylamide (BANA) test was used for the microbiological analysis. Clinical parameters from baseline to 6 months at different time intervals demonstrated a significant reduction in mean GI, PI, GBI, and PPD and gain in CAL and RAL over a period of 6 months, and results were statistically significant in all the three treatment groups. However, on intergroup comparison, clinically significant difference was observed, but statistically, results were significant at few intervals. The microbiological analysis revealed a statistically nonsignificant reduction at the end of 6 months on intra- and intergroup comparison. The study concluded that the use of *A. catechu* gel and *A. oleracea* gel clinically showed improvement in clinical parameters when combined with SRP, attributing to better anti-inflammatory and healing properties.

Key words: Acacia catechu gel, Acmella oleracea gel, Chronic periodontitis, Clinical attachment level, Gingival index, Local drug delivery

Introduction

A thorough understanding of the etiopathogenesis of periodontal disease has laid out the clinicians and researchers with various examination tools and procedures that extended the therapy alternative. Dr. Goodson *et al.* in 1979 initiated the idea of standard deliverance of medicaments for periodontal therapy. Since then, various investigations have been done throughout the years with numerous antimicrobial agents in different clinical scenarios [1].

Local drug delivery (LDD) of antimicrobial treatment to periodontal pockets has the benefit of administering more drugs at the target site while minimizing the exposure of the whole body to drug and sustained release of agent in periodontal pocket [2]. Several LDD formulations include tetracycline fibers, doxycycline polymer, minocycline biodegradable ointment and gel, and metronidazole as a biodegradable gel [3-5]. There are many herbs which have been incorporated for use as oral medicaments, but so far,

two herbs, i.e., *Acmella oleracea* and *Acacia catechu*, have not yet been tested as LDD despite their known beneficial properties. *A. catechu*, also known as black khair, belongs to family: Fabaceae [6-10]. *A. oleracea* is a plant species of Asteraceae family extensively found in parts of America, North Australia, Africa, Malaya, Borneo, India, and Sri Lanka [11-13].

Phytochemicals are the main substance in herbs having defensive and interceptive action against diseases. This plant is used for its medicinal purposes for the treatment of gout, GIT diseases, malaria and also used as a cosmetic plant [14-17]. The extract of the herbs is also useful in dental conditions; *Spilanthes acmella* often called as anti-toothache plant is used to stunt pain in throat problems, tooth, or paralysis of the tongue; the people chew the pungent flower heads of the plant [18-20].

Jahan *et al.* described that *S. acmella*'s ethanolic concentrate has antimicrobial action against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus*

pyogenes, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* [21]. The extract of the plant also had antifungal properties, especially for its use in treatment of periodontal disease [22, 23]. Herbal medicines have been widely used all over the world for being nontoxic and compatible and have gained momentum toward deployment of natural substances for their use in dentistry [24].

The aim of this study was to evaluate and compare the efficacy of locally delivered *A. oleracea* and *A. catechu* gels in the management of chronic generalized periodontitis: a clinical and microbiological study.

Aim and objectives

The aim of the study was to evaluate the efficacy of locally delivered *A. oleracea* and *A. catechu* gels in the management of generalized chronic periodontitis: a clinical and microbiological study and the objectives were to evaluate the effect of the herbal gels on the gingival status based on clinical features and to evaluate the effect of the herbal gels on subgingival microflora.

Materials and Methods

A 6-month randomized control clinical trial was performed with a split-mouth design with ninety sites. Thirty patients diagnosed with chronic generalized periodontitis aged 25–65 years and willing to comply with the oral hygiene instructions and report for required number of recall visits having pocket probing depth (PPD) of ≥ 5 mm were included. Smokers, expecting or nursing mothers, patients with altered immune system, and those who are mentally and physically challenged were excluded from the study. Informed consent was obtained on the day of treatment before the procedure; a short case history including specific clinical parameters such as gingival index (GI), plaque index (PI), gingival bleeding index (GBI), PPD, clinical attachment level (CAL), and relative attachment level (RAL) was recorded using UNC-15 probe (**Figures 1a-1c and 2a-2c**). The microbiological parameter, i.e., N-benzoyl-DL-arginine-2-naphthylamide (BANA) test, was done before the placement of gel at baseline and post the placement of gel at 6 months (**Figures 1d, 1e, 2d and 2e**). Subgingival plaque samples from selected sites were analyzed for periodontopathic anaerobic microorganisms by using BANA reagent strips (London, United Kingdom) (BANA Met LLC, Ann Arbor, Michigan, USA). After sampling the desired site, the upper portion of the matrix strip with the reagent was moistened with distilled water using an autoclaved sterile cotton pellet which was folded and placed in an incubator for 15 min at 55°C.



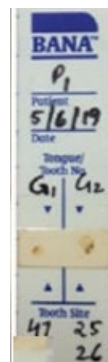
a)



b)



c)



d)



e)

Figure 1. (a) Probing depth at baseline. (b) Placement of *Acmella oleracea* gel. (c) Probing depth at 6 months. (d) N-benzoyl-DL-arginine-2-naphthylamide-(BANA) test at baseline. (e) N-benzoyl-DL-arginine-2-naphthylamide-Zyme™ test at 6 months



a)

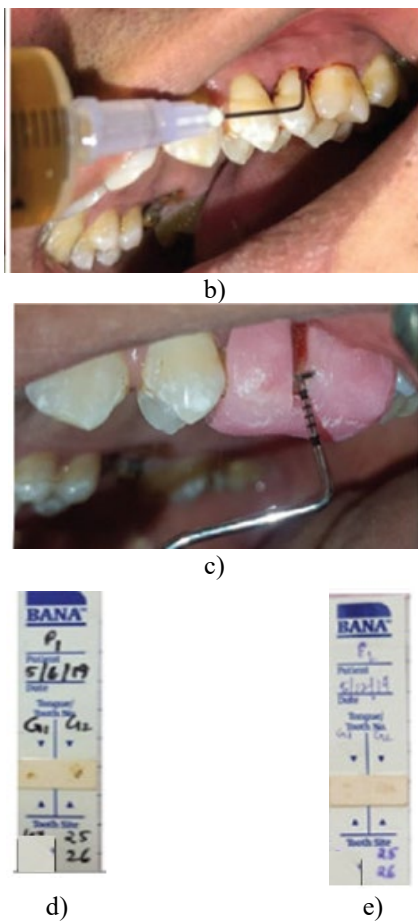


Figure 2. (a) Probing depth at baseline. (b) Placement of *Acacia catechu* gel. (c) Probing depth at 6 months. (d) N-benzoyl-DL-arginine-2-naphthylamide-(BANA) test at baseline. (e) N-benzoyl-DL-arginine-2-naphthylamide-Zyme™ test at 6 months

A custom-made acrylic stent was used to standardize the measurement of clinical parameters. With the aid of curette, subgingival samples were taken from the pocket before scaling and root planing (SRP). The sites were divided into three different quadrants having a PPD 5–7 mm. SRP was performed at the control and two test sites. The placement of gel containing *A. oleracea* (1%) (Group 1) and *A. catechu* (1%) (Group 2) as LDD was placed at the two test sites, respectively. Methyl paraben as a specific component has a shelf life of 5 years, and hence for the shelf life of herbal gel, 3 years is considered. The gels had a sustained release pattern and they were not reinserted after baseline as this is the first study for *A. oleracea* and *A. catechu* gel for the treatment of chronic periodontitis. Periodontal dressing was applied to all the three sites after procedure, and patients were given oral care guidelines. The patients were recalled after 7 days for dressing removal and follow-up after 1 month, 3 months, and 6 months for recording the clinical parameters and 6 months for recording microbiological parameters, respectively.

Fresh plants *A. oleracea* (Figure 3c) and *A. catechu* (Figure 3a) were collected from the Raiwala district, Uttarakhand.

The plants were identified and authenticated by the Senior Scientist (Taxonomist), NBPGR, Pusa Campus, New Delhi. The numbers were allotted accordingly for the voucher specimen. Plant parts were dried (Figures 3b and 3d) and then grounded using mechanical grinder to coarse powder. Accurately weighed carboxymethylcellulose (CMC) sodium 5 g was transferred in a beaker with 100-ml distilled water and stirred thoroughly over 80°C–90°C over a Bunsen burner; preservatives 0.2 g propyl paraben and 0.02 g methyl paraben were added and continuously stirred to get a clear suspended solution. After the solution was cooled down, 1 g of extract of *A. catechu* was added to 100 ml of CMC solution and mixed vigorously to attain uniform gel consistency of 1% w/v. The gel was later transferred to a plastic container and ready for LDD purpose.





d)

Figure 3. Clinical parameter scores and TNF- α Levels around implant. PI; PBI; PD; TNF- α ; (a-d) PI, PBI, PD, and TNF- α levels between wide and narrow keratinized tissue around implant. NS $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, by Independent t -test analysis. TNF- α – Tumour necrosis factor alpha, PI – Plaque index, PBI – Papilla bleeding index, PD – Pocket depth, NS – Nonsignificant

The prepared gel was loaded into an insulin syringe and delivered into the pocket with gentle pressure covering the depths and curves of the pocket site. To close the entrance of the gingival margin, the gingiva was carefully adapted with digital pressure and Coe-Pak was placed.

Results and Discussion

The statistical assessment was done using the SPSS (Statistical package for social sciences) analysis software version 17. Paired and independent Student's t -test, one-way analysis of variance test, and *post hoc* Bonferroni test for correlation coefficient were used to derive the result. For this study, the total sample size was calculated by using $n = 4 \cdot \sigma^2 / I^2$ by putting standard deviation (σ) = 0.4150 and least permissible error (I) = 0.0755 (i.e., power of study 93%), respectively, after putting the all of these values. In the above-said formula, the sample of size 30 was obtained. Further, $P < 0.05$ was considered statistically significant ($P < 0.05$) at 0.05 level of significance. Significant reduction in PI, GI, GBI, and PDD from baseline, 1 month, 3 months, and 6 months was observed in all the three groups (**Tables 1-6, Figure 4**). Both test groups revealed a decrease in PPD with a noticeable reduction in Group 2 as compared to Group 1 and Group 3 (**Tables 7 and 8**). Furthermore, a marked gain in CAL and RAL was seen in Group 2 than Group 1 and Group 3 between the baseline and follow-up periods (**Tables 9-12**). However, these parameters were statistically insignificant but clinically significant in Group 1 and Group 3. The control group and both test groups on intra- and intergroup comparisons for microbiological analysis revealed a statistically nonsignificant reduction at the end of 6 months (**Table 13, Figure 5**).

Table 1. Intergroup comparison of the mean gingival index at baseline, 1 month, 3 months, and 6 months

GI	Group no	Group no	MD	P
Baseline–1 month	Group 1	Group 2	0.07	0.483
	Group 1	Group 3	0.07	0.483
	Group 2	Group 3	0.00	1.000
Baseline–3 months	Group 1	Group 2	-0.13	0.663
	Group 1	Group 3	-0.03	1.000
	Group 2	Group 3	0.10	1.000
Baseline–6 months	Group 1	Group 2	-0.13	1.000
	Group 1	Group 3	-0.20	0.529
	Group 2	Group 3	-0.07	1.000
1 month–3 months	Group 1	Group 2	-0.20	0.210
	Group 1	Group 3	-0.10	1.000
	Group 1	Group 3	0.10	1.000
1 month–6 months	Group 1	Group 2	-0.20	0.496
	Group 1	Group 3	-0.27	0.196
	Group 2	Group 3	-0.07	1.000
3 months–6 months	Group 1	Group 2	0.00	1.000
	Group 1	Group 3	-0.17	0.653
	Group 2	Group 3	-0.17	0.653

Post hoc Bonferroni test. # Nonsignificant difference. The intergroup comparison of mean GI at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. No significant difference was found for the intergroup comparisons. GI – Gingival index; MD – Mean difference; P – Probability value; P value < 0.05; P value is significant

Table 2. Intragroup comparison for the mean gingival index at baseline, 1 month, 3 months, and 6 months

GI	Group no	Mean	SD	FV	P
Baseline	Group 1	2.40	0.50	0.139	0.870
	Group 2	2.33	0.48		
	Group 3	2.37	0.49		
1 month	Group 1	1.37	0.49	0.046	0.955
	Group 2	1.37	0.49		
	Group 3	1.40	0.50		
3 months	Group 1	1.23	0.43	2.578	0.082
	Group 2	1.03	0.18		
	Group 3	1.17	0.38		
6 months	Group 1	0.57	0.50	1.979	0.144
	Group 2	0.37	0.49		
	Group 3	0.33	0.48		
Baseline–1 month	Group 1	1.03	0.18	1.333	0.269
	Group 2	0.97	0.18		
	Group 3	0.97	0.18		
Baseline–3 months	Group 1	1.17	0.38	0.823	0.442
	Group 2	1.30	0.47		
	Group 3	1.20	0.41		
Baseline–6 months	Group 1	1.83	0.53	0.963	0.386
	Group 2	1.97	0.61		
	Group 3	2.03	0.56		
1 month–3 months	Group 1	0.13	0.35	1.684	0.192
	Group 2	0.33	0.48		
	Group 3	0.23	0.43		
1 month–6 months	Group 1	0.80	0.55	1.885	0.158
	Group 2	1.00	0.59		
	Group 3	1.07	0.52		
3 months–6 months	Group 1	0.67	0.48	1.028	0.362
	Group 2	0.67	0.48		
	Group 3	0.83	0.59		

One-way ANOVA test. # Nonsignificant difference. The mean GI for baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared using the one-way ANOVA test among Group 1, Group 2, and Group 3. The mean GI discrepancy for baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was not significant. *P* value significant ($P \leq 0.05$). GI – Gingival index; SD – Standard deviation; FV – *F*-value; *P* – Probability value

Table 3. Intergroup comparison of the plaque index at baseline, 1 month, 3 months, and 6 months

PI	Group no	Group no	MD	P
Baseline–1 month	Group 1	Group 2	0.07	0.918
	Group 1	Group 3	0.04	1.000
	Group 2	Group 3	–0.03	1.000
Baseline–3 months	Group 1	Group 2	–0.05	1.000
	Group 1	Group 3	0.14	0.705
	Group 2	Group 3	0.19	0.318
Baseline–6 months	Group 1	Group 2	–0.16	0.847
	Group 1	Group 3	0.23	0.347
	Group 2	Group 3	0.39	0.027*
1 month–3 months	Group 1	Group 2	–0.12	0.878
	Group 1	Group 3	0.10	1.000
	Group 2	Group 3	0.21	0.168

1 month–6 months	Group 1	Group 2	–0.22	0.381
	Group 1	Group 3	0.19	0.580
	Group 2	Group 3	0.41	0.016*
3 months–6 months	Group 1	Group 2	–0.11	1.000
	Group 1	Group 3	0.09	1.000
	Group 2	Group 3	0.20	0.458

Post hoc Bonferroni test. *Significant difference. The intergroup comparison of mean PI at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. No significant difference was found for the intergroup comparisons. The mean PI at 6 months was significantly more among Groups 1 and 3 compared to Group 2. The mean PI at baseline–6 months and 1 month–6 months was significantly more among Group 2 compared to Group 3. *P* value significant ($P \leq 0.05$). PI – Plaque index; MD – Mean difference; *P* – Probability value

Table 4. Intragroup comparison for the mean plaque index at baseline, 1 month, 3 months, and 6 months

PI	Group no	Mean	SD	FV	<i>P</i>
Baseline	Group 1	2.55	0.42	2.618	0.079
	Group 2	2.33	0.51		
	Group 3	2.26	0.57		
1 month	Group 1	1.93	0.47	1.498	0.229
	Group 2	1.78	0.55		
	Group 3	1.68	0.64		
3 months	Group 1	1.32	0.52	2.112	0.127
	Group 2	1.05	0.53		
	Group 3	1.17	0.46		
6 months	Group 1	0.56	0.54	4.901	0.010*
	Group 2	0.18	0.35		
	Group 3	0.50	0.57		
Baseline–1 month	Group 1	0.62	0.25	0.537	0.586
	Group 2	0.55	0.27		
	Group 3	0.58	0.23		
Baseline–3 months	Group 1	1.23	0.55	1.430	0.245
	Group 2	1.28	0.41		
	Group 3	1.10	0.34		
Baseline–6 months	Group 1	1.99	0.60	3.608	0.031*
	Group 2	2.15	0.57		
	Group 3	1.76	0.51		
1 month–3 months	Group 1	0.61	0.50	1.880	0.159
	Group 2	0.73	0.38		
	Group 3	0.52	0.38		
1 month–6 months	Group 1	1.37	0.57	4.073	0.020*
	Group 2	1.60	0.60		
	Group 3	1.18	0.52		
3 months–6 months	Group 1	0.76	0.51	1.043	0.357
	Group 2	0.87	0.61		
	Group 3	0.67	0.48		

One-way ANOVA test. *Significant difference. The mean PI at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared among Group 1, Group 2, and Group 3 with the help of one-way ANOVA test. There was a significant difference in mean PI at baseline–6 months and 1 month–6 months among Group 1, Group 2, and Group 3. *P* value significant ($P \leq 0.05$). PI – Plaque index; SD – Standard deviation; FV – *F*-value; *P* – Probability value

Table 5. Intergroup comparison of the mean gingival bleeding index at baseline, 1 month, 3 months, and 6 months

GBI	Group no	Group no	MD	<i>P</i>
Baseline–1 month	Group 1	Group 2	8.30	0.027*

Baseline–3 months	Group 1	Group 3	–2.40	1.000
	Group 2	Group 3	–10.70	0.003*
	Group 1	Group 2	7.92	0.018*
Baseline–6 months	Group 1	Group 3	–1.15	1.000
	Group 2	Group 3	–9.07	0.005*
	Group 1	Group 2	6.90	0.026*
1 month–3 months	Group 1	Group 3	–3.02	0.728
	Group 2	Group 3	–9.92	0.001*
	Group 1	Group 2	–0.38	1.000
1 month–6 months	Group 1	Group 3	1.25	1.000
	Group 2	Group 3	1.63	1.000
	Group 1	Group 2	–1.40	1.000
3 months–6 months	Group 1	Group 3	–0.62	1.000
	Group 2	Group 3	0.78	1.000
	Group 1	Group 2	–1.02	1.000
	Group 1	Group 3	–1.87	1.000
	Group 2	Group 3	–0.85	1.000

Post hoc Bonferroni test. *Significant difference. The intergroup comparison of mean GBI at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. No significant difference was found for the intergroup comparisons. The mean GBI at baseline–1 month, baseline–3 months, and baseline–6 months was clinically significant, with Groups 1 and 3 showing better results compared to Group 2. However, statistically intergroup comparison showed no significant difference. *P* value significant ($P \leq 0.05$). GBI – Gingival bleeding index; MD – Mean difference; *P* – Probability value

Table 6. Intragroup comparison for the mean gingival bleeding index at baseline, 1 month, 3 months, and 6 months

GBI	Group no	Mean	SD	FV	<i>P</i>
Baseline	Group 1	83.13	10.58	2.013	0.055
	Group 2	76.83	8.59		
	Group 3	85.50	8.46		
1 month	Group 1	37.91	12.20	0.274	0.761
	Group 2	39.92	12.79		
	Group 3	37.88	11.58		
3 months	Group 1	21.51	8.61	0.313	0.732
	Group 2	23.13	7.78		
	Group 3	22.73	8.36		
6 months	Group 1	14.53	3.57	0.767	0.468
	Group 2	15.13	4.51		
	Group 3	13.88	3.58		
Baseline–1 month	Group 1	45.22	12.59	6.513	0.002*
	Group 2	36.92	14.82		
	Group 3	47.62	7.58		
Baseline–3 months	Group 1	61.62	13.92	6.144	0.003*
	Group 2	53.70	10.61		
	Group 3	62.77	7.10		
Baseline–6 months	Group 1	68.60	11.38	7.844	0.001*
	Group 2	61.70	10.01		
	Group 3	71.62	8.16		
1 month–3 months	Group 1	16.40	12.50	0.201	0.818
	Group 2	16.78	9.99		
	Group 3	15.15	8.37		
1 month–6 months	Group 1	23.38	12.18	0.098	0.907
	Group 2	24.78	13.10		
	Group 3	24.00	11.54		
3 months–6 months	Group 1	6.98	8.23	0.375	0.689

Group 2	8.00	9.20
Group 3	8.85	7.64

One-way ANOVA test. *Significant difference. The mean GBI at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared among Group 1, Group 2, and Group 3 using the one-way ANOVA test. There was a significant difference in mean GBI at baseline–1 month, baseline–3 months, and baseline–6 months among Group 1, Group 2, and Group 3. *P* value significant ($P \leq 0.05$). GBI – Gingival bleeding index; SD – Standard deviation; FV – *F*-value; *P* – Probability value

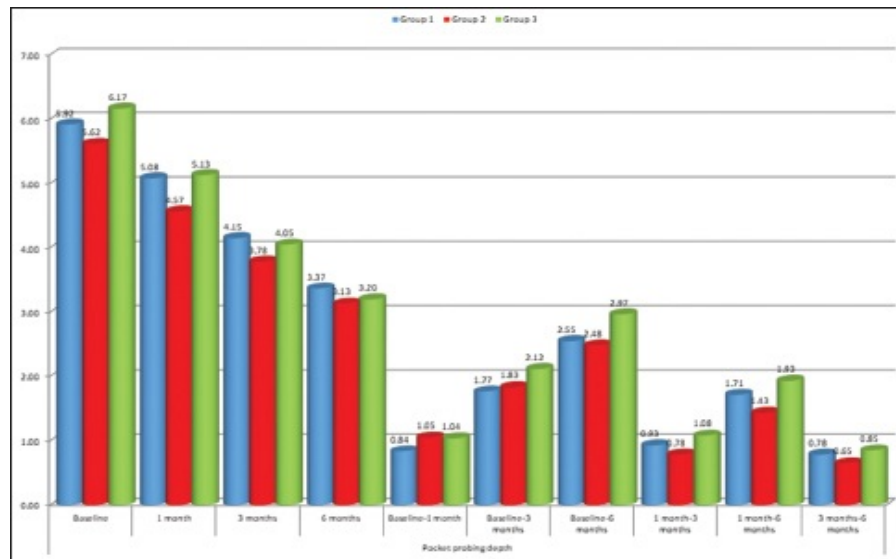


Figure 4. Pocket probing depth at different time intervals at baseline, 1 month, 3 months, and 6 months

Table 7. Intergroup comparison of mean pocket probing depth at baseline, 1 month, 3 months, and 6 months

PPD	Group no	Group no	MD	<i>P</i>
Baseline–1 month	Group 1	Group 2	–0.04	1.000
	Group 1	Group 3	–0.37	0.236
	Group 2	Group 3	–0.15	1.000
Baseline–3 months	Group 1	Group 2	0.22	0.870
	Group 1	Group 3	–0.55	0.027*
	Group 2	Group 3	–0.13	1.000
Baseline–6 months	Group 1	Group 2	0.42	0.138
	Group 1	Group 3	–0.77	0.001*
	Group 2	Group 3	–0.41	0.115
1 month–3 months	Group 1	Group 2	0.77	0.001*
	Group 1	Group 3	0.36	0.213
	Group 2	Group 3	–0.18	0.884
1 month–6 months	Group 1	Group 2	0.02	1.000
	Group 1	Group 3	0.18	0.884
	Group 2	Group 3	0.19	0.755
3 months–6 months	Group 1	Group 2	–0.39	0.048*
	Group 1	Group 3	–0.26	0.440
	Group 2	Group 3	0.39	0.048*

Post hoc Bonferroni test. *Significant difference. The intergroup comparison of mean PPD at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. The mean PPD at baseline–3 months, baseline–6 months, and 3 months–6 months was clinically significant, with Group 2 showing better results as compared to Groups 1 and 3. However, statistically intergroup comparison showed no significant difference. *P* value significant ($P \leq 0.05$). PPD – Pocket probing depth; MD – Mean difference; *P* – Probability value

Table 8. Intragroup comparison for the mean pocket probing depth at baseline, 1 month, 3 months, and 6 months

PPD	Group no	Mean	SD	FV	P
Baseline	Group 1	5.52	0.90	2.540	0.062
	Group 2	6.25	0.84		
	Group 3	5.93	0.60		
1 month	Group 1	4.19	0.87	1.414	0.249
	Group 2	4.55	0.88		
	Group 3	4.46	0.83		
3 months	Group 1	3.32	0.53	2.254	0.111
	Group 2	3.50	0.43		
	Group 3	3.60	0.60		
6 months	Group 1	2.76	0.43	0.095	0.910
	Group 2	2.72	0.36		
	Group 3	2.76	0.35		
Baseline–1 month	Group 1	1.32	0.94	1.604	0.207
	Group 2	1.70	0.83		
	Group 3	1.47	0.64		
Baseline–3 months	Group 1	2.20	0.90	3.882	0.024*
	Group 2	2.75	0.87		
	Group 3	2.33	0.58		
Baseline–6 months	Group 1	2.76	0.85	7.733	0.001*
	Group 2	3.53	0.83		
	Group 3	3.17	0.55		
1 month–3 months	Group 1	0.87	0.59	0.818	0.445
	Group 2	1.05	0.73		
	Group 3	0.86	0.63		
1 month–6 months	Group 1	1.44	0.63	2.538	0.085
	Group 2	1.83	0.72		
	Group 3	1.70	0.70		
3 months–6 months	Group 1	0.56	0.28	4.897	0.010*
	Group 2	0.78	0.35		
	Group 3	0.84	0.44		

One-way ANOVA test. *Significant difference. The mean PPD at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared among Group 1, Group 2, and Group 3 using the one-way ANOVA test. There was a significant difference in mean PPD at baseline–3 months, baseline–6 months, and 3 months–6 months among Group 1, Group 2, and Group 3. *P* value significant ($P \leq 0.05$). PPD – Pocket probing depth; SD – Standard deviation; FV – *F*-value; *P* – Probability value

Table 9. Intergroup comparison of the mean clinical attachment level at baseline, 1 month, 3 months, and 6 months

CAL (mm)	Group no	Group no	MD	P
Baseline–1 month	Group 1	Group 2	–0.42	0.264
	Group 1	Group 3	–0.20	1.000
	Group 2	Group 3	0.22	1.000
Baseline–3 months	Group 1	Group 2	–0.52	0.042*
	Group 1	Group 3	–0.09	1.000
	Group 2	Group 3	0.43	0.045*
Baseline–6 months	Group 1	Group 2	–0.83	0.002*
	Group 1	Group 3	–0.51	0.048*
	Group 2	Group 3	0.31	0.539
1 month–3 months	Group 1	Group 2	–0.10	1.000
	Group 1	Group 3	0.11	1.000
	Group 2	Group 3	0.21	0.744

1 month–6 months	Group 1	Group 2	–0.40	0.092
	Group 1	Group 3	–0.31	0.284
	Group 2	Group 3	0.09	1.000
3 months–6 months	Group 1	Group 2	–0.30	0.025*
	Group 1	Group 3	–0.42	0.001*
	Group 2	Group 3	–0.12	0.863

Post hoc Bonferroni test. *Significant difference. The intergroup comparison of mean CAL (mm) at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. The mean CAL (mm) at baseline–3 months, baseline–6 months, and 3 months–6 months was clinically significant, with Group 2 showing better results as compared to Groups 1 and 3. However, statistically intergroup comparison showed no significant difference. *P* value significant ($P \leq 0.05$). CAL – Clinical attachment level; MD – Mean difference; *P* – Probability value

Table 10. Intragroup comparison for the mean clinical attachment level at baseline, 1 month, 3 months, and 6 months

CAL (mm)	Group no	Mean	SD	FV	<i>P</i>
Baseline	Group 1	7.52	1.14	2.120	0.058
	Group 2	8.25	0.84		
	Group 3	7.95	0.62		
1 month	Group 1	6.24	0.87	1.039	0.358
	Group 2	6.55	0.88		
	Group 3	6.47	0.84		
3 months	Group 1	5.29	0.51	3.325	0.041*
	Group 2	5.50	0.43		
	Group 3	5.64	0.60		
6 months	Group 1	4.82	0.46	0.515	0.600
	Group 2	4.72	0.36		
	Group 3	4.74	0.32		
Baseline–1 month	Group 1	1.27	1.25	1.491	0.231
	Group 2	1.70	0.83		
	Group 3	1.48	0.67		
Baseline–3 months	Group 1	2.22	1.22	2.649	0.046*
	Group 2	2.75	0.87		
	Group 3	2.31	0.64		
Baseline–6 months	Group 1	2.70	1.17	6.488	0.002*
	Group 2	3.53	0.83		
	Group 3	3.21	0.60		
1 month–3 months	Group 1	0.95	0.68	0.677	0.511
	Group 2	1.05	0.73		
	Group 3	0.84	0.73		
1 month–6 months	Group 1	1.43	0.67	2.651	0.076
	Group 2	1.83	0.72		
	Group 3	1.74	0.74		
3 months–6 months	Group 1	0.48	0.43	7.566	0.001*
	Group 2	0.78	0.35		
	Group 3	0.90	0.51		

One-way ANOVA test. *Significant difference. The mean CAL (mm) at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared among Group 1, Group 2, and Group 3 using the one-way ANOVA test. There was a significant difference in mean CAL (mm) at 1 month, 3 months, 6 months, baseline–6 months, and 1 month–6 months among Group 1, Group 2, and Group 3. *P* value significant ($P \leq 0.05$). CAL – Clinical attachment level; SD – Standard deviation; FV – *F*-value; *P* – Probability value

Table 11. Intergroup comparison of relative attachment level at baseline, 1 month, 3 months, and 6 months

RAL (mm)	Group no	Group no	MD	<i>P</i>
Baseline–1 month	Group 1	Group 2	–0.23	0.144
	Group 1	Group 3	–0.12	0.957

Baseline–3 months	Group 2	Group 3	0.12	0.957
	Group 1	Group 2	–0.23	0.472
	Group 1	Group 3	–0.30	0.198
Baseline–6 months	Group 2	Group 3	–0.07	1.000
	Group 1	Group 2	0.00	1.000
	Group 1	Group 3	–0.18	0.038*
1 month–3 months	Group 2	Group 3	–0.19	0.032*
	Group 1	Group 2	0.05	1.000
	Group 1	Group 3	–0.18	1.000
1 month–6 months	Group 2	Group 3	–0.23	0.804
	Group 1	Group 2	0.05	1.000
	Group 1	Group 3	–0.08	1.000
3 months–6 months	Group 2	Group 3	–0.13	0.990
	Group 1	Group 2	–0.18	0.047*
	Group 1	Group 3	–0.20	0.046*
	Group 2	Group 3	–0.02	1.000

Post hoc Bonferroni test. *Significant difference. The intergroup comparison of mean RAL (mm) at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was done using the *post hoc* Bonferroni test. The mean RAL (mm) at 1 month–3 months was clinically significant, with Group 2 showing better results as compared to Groups 1 and 3. However, statistically intergroup comparison showed no significant difference. *P* value significant ($P \leq 0.05$). RAL – Relative attachment level; MD – Mean difference; *P* – Probability value

Table 12. Intragroup comparison for the mean relative attachment level at baseline, 1 month, 3 months, and 6 months

RAL (mm)	Group no	Mean	SD	FV	<i>P</i>
Baseline	Group 1	8.35	1.13	1.598	0.208
	Group 2	8.48	1.32		
	Group 3	8.92	1.38		
1 month	Group 1	7.57	0.86	1.681	0.192
	Group 2	7.75	1.07		
	Group 3	8.05	1.14		
3 months	Group 1	7.18	0.79	1.792	0.173
	Group 2	7.13	1.04		
	Group 3	7.55	0.94		
6 months	Group 1	6.23	0.73	0.695	0.502
	Group 2	6.19	0.81		
	Group 3	6.42	0.85		
Baseline–1 month	Group 1	0.78	0.60	0.490	0.614
	Group 2	0.73	0.45		
	Group 3	0.87	0.52		
Baseline–3 months	Group 1	1.17	0.67	0.926	0.400
	Group 2	1.35	0.53		
	Group 3	1.37	0.68		
Baseline–6 months	Group 1	2.12	0.76	2.270	0.127
	Group 2	2.30	0.69		
	Group 3	2.50	0.72		
1 month–3 months	Group 1	0.38	0.45	2.209	0.044*
	Group 2	0.62	0.43		
	Group 3	0.50	0.47		
1 month–6 months	Group 1	1.33	0.66	1.895	0.156
	Group 2	1.56	0.57		
	Group 3	1.63	0.64		
3 months–6 months	Group 1	0.95	0.40	2.140	0.024*
	Group 2	0.95	0.45		

Group 3 1.13 0.35

One-way ANOVA test. *Significant difference. The mean RAL (mm) at baseline, 1 month, 3 months, 6 months, baseline–1 month, baseline–3 months, baseline–6 months, 1 month–3 months, 1 month–6 months, and 3 months–6 months was compared among Group 1, Group 2, and Group 3 with the help of one-way ANOVA test. There was a significant difference in mean RAL (mm) at 1 month and 1 month–3 months among Group 1, Group 2, and Group 3. *P* value significant ($P \leq 0.05$). RAL – Relative attachment level; SD – Standard deviation; FV – *F*-value; *P* – Probability value

Table 13. Distribution of the microbiological analysis at baseline and 6 months

Microbiological analysis	Groups			χ^2	<i>P</i>
	Group 1	Group 2	Group 3		
Baseline					
Positive	10 (33.3)	21 (70.0)	13 (43.3)	2.626	0.053
WP	20 (66.7)	9 (30.0)	17 (56.7)		
6 months					
Negative	26 (86.7)	26 (86.7)	25 (83.3)	0.180	0.914
WP	4 (13.3)	4 (13.3)	5 (16.7)		

Chi-square test. # Nonsignificant difference. The distribution of microbiological analysis at baseline and 6 months was compared between Group 1, Group 2, and Group 3 using the Chi-square test. There was no significant difference in the distribution of microbiological analysis at baseline and 6 months among Group 1, Group 2, and Group 3. WP – Weak positive; χ^2 – Chi square value; *P* – Probability value; *P* value < 0.05; *P* value is significant

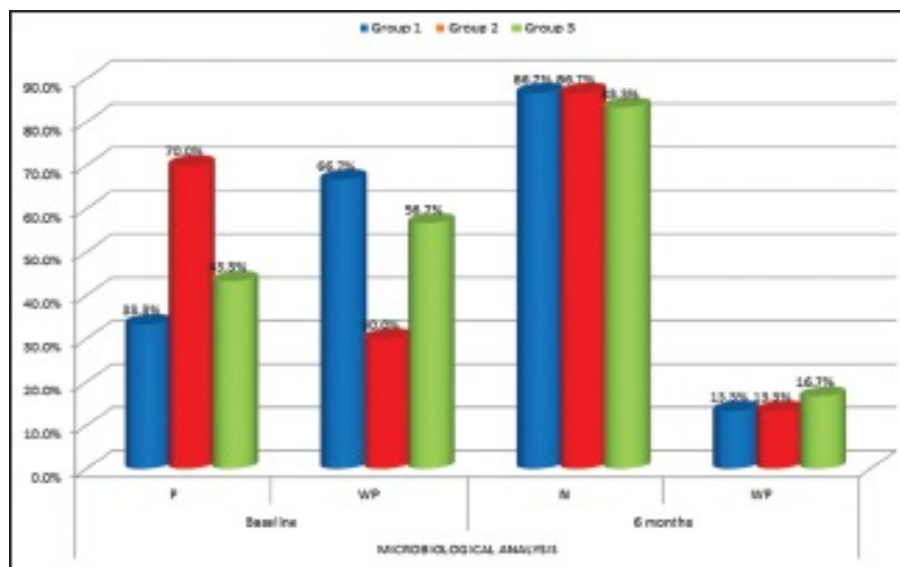


Figure 5. Microbiological parameter at baseline and 6 months

The results showed that application of the herbal extracts of *A. oleracea* and *A. catechu* as LDD improved the periodontal healing compared to the control group with only SRP, thus suggesting that active herbal ingredients play an important role in this effect. The anti-bactericidal role of *S. acmella* reduces bacterial adherence to tooth surface and their growth rate [25, 26]. LDD of antimicrobial agents into periodontal pocket has been widely developed and investigated since the 1970s. Regulated release systems have received interest in periodontal therapy and seem to have some promises. The relative existence of herbal extracts is a common type of alternative and complementary medicine promising path to multiple oral infection prevention and therapeutic strategies [27, 28].

The phytochemical constituents of *A. catechu* will help analyze the various medicinal properties such as anti-inflammatory and anti-bactericidal and play a role in

effective wound healing activities [29, 30]. Significant probing pocket depth reduction and CAL gain were observed with adjunctive use of these herbal gels. The secondary parameters of plaque control, gingival healing, and GBI showed better outcomes in the gel group compared to SRP alone.

A study on *A. catechu* by Lakshmi reported a 70%–72% reduction in GI scores during the 15 days of the study period [31]. A similar study by Pradeep *et al.* on the prescription gel containing *Acacia arabica* easily available in markets showed to be effective in decreasing plaque and gingival inflammation in subjects with gingivitis when compared to chlorhexidine [32–34].

Significant reduction in the PPD and gain in CAL with the use of *A. arabica* gum as an adjunct to SRP in the treatment of chronic periodontitis were reported [35–37].

In this research, the BANA-Enzymatic microbiological assessment displayed a significant drop in microbial load from baseline to 6 months. Similarly, an investigation was performed by Dhalla *et al.* for the presence of BANA microorganisms in adult periodontitis before and after BANA-Enzymatic™ test kit SRP: an *in vivo* study showed that the BANA tests were correlated statistically with the risk of destruction of periodontium [38].

Many herbs have shown remarkable medicinal properties, but only a few have been approved due to the lack of a randomized controlled clinical trial. In order to understand these herbs in detail, several studies are currently being conducted.

Burnett *et al.* studied a unique suspension of extracts of *A. catechu* by *Scutellaria baicalensis* for its effectiveness at inhibiting the activities of 5-lipoxygenase and cyclooxygenase enzymes *in vitro*, cellular, and *in vivo* models [39]. A study was conducted to evaluate the anti-inflammatory activity of *S. acmella* consisting spilanthol by downregulating inflammatory mediators on murine macrophage induced by LPS probably due to the NF-kappa B inactivity, which adversely regulated the development of pro-inflammatory mediators [40].

From the results of this study, both *A. oleracea* and *A. catechu* could be used as an adjunct to SRP. This combination would enhance wound healing properties. However, further studies with microbiological assessments are necessary for more meaningful results.

Future recommendations were as follows:

- Long-term longitudinal studies with larger sample size are recommended for more meaningful results
- Advance microbiological analyses with polymerase chain reaction are recommended for both qualitative and quantitative assessments.

Conclusion

The following conclusions could be drawn from this study:

- All the three groups demonstrated improvements in the clinical parameters GI, PI, GBI, PPD, CAL, and RAL
- With respect to PPD, CAL, and RAL, *A. catechu* gel and *A. oleracea* gel showed better results than SRP alone but statistically nonsignificant. However, *A. oleracea* gel had more clinically significant results in comparison with *A. catechu*, but not statistically significant
- The microbiological analysis revealed a statistically nonsignificant reduction in both test groups and control group at the end of 6 months on both intra- and intergroup comparisons.

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