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Original Article

DEVELOPMENT, FORMULATION, AND EVALUATION OF *ALOE VERA* TOOTH GEL: AN ANTIMICROBIAL STUDY

ABU SHOEB, ANANDAMOY RUDRA*

***Department of Pharmaceutics, Bengal School of Technology, Sugandha, Delhi Road, Hooghly-712102, West Bengal, India**
Email: anandaju05@gmail.com

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ABSTRACT

Objective: The purpose of the current study was to develop and formulate tooth gel using *Aloe vera* leaf extract and evaluate. The experiment was designed to provide scientific proof of the antimicrobial activity of *Aloe vera* (*Aloe barbadensis Mill*) in tooth gel formulation against bacteria Staphylococcus *aureus* which causes infections associated with dental caries.

Methods: Transparent *Aloe vera* gel extract was consistently blended for five minutes at 1000 Rotations Per Minute (RPM). Carbopol 940 and Carboxy Methyl Cellulose (CMC) were used as excipients in the formulation of *Aloe vera* tooth gel.

Results: The formulated *Aloe vera* tooth gel was evaluated by physical examination such as color (yellowish green), good homogeneity and smoothness. pH and viscosity of developed tooth gel preparation were found to be 7.9 and 4.9 Pa. S respectively. The developed *Aloe vera* tooth gel showed considerable effectiveness with a Zone of Inhibition (ZOI) of 0.022 m, according to an antimicrobial study against *Staphylococcus aureus*. A comparison between formulated gel and marketed products (Colgate Natural Extract *Aloe vera,* Himalaya Herbal Active Fresh, Dabur Red) was also carried out.

Conclusion: The formulated herbal tooth gel exhibited antimicrobial activity against gram-positive bacteria *Staphylococcus aureus*. The developed formulation (F4) with the ZOI of 0.022 m could be comparable with the marketed product.

Keywords: *Aloe vera,* Tooth gel, Attenuated total reflectance (ATR), Antimicrobial study, *Staphylococcus aureus,* Dental caries

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INTRODUCTION

There has been increasing attention paid in recent years to oral infectious diseases and oral inflammations, in particular, infectious dental diseases. Dental infections, other wisely known as periodontitis, are a group of infections, predominantly caused by the colonization of gram-negative, anaerobic pathogens on sub-gingival areas [1, 2].

The *Aloe* plant is grown in warm tropical areas and cannot survive freezing temperatures. There are many species of *Aloe* grown around the world but the main medicinal one is *Aloe vera* Barbadensis [3, 4]. *Aloe vera*, a very popular herbal remedy, which is used medicinally for thousands of years, has multiple dental uses. It has been shown to enhance defense mechanisms and has a variety of components to help combat periodontal diseases and other oral conditions. In multiple clinical studies, *Aloe vera* has used in dentistry for wound-healing effect, gingivitis, plaque control and curing oral mucosal lesions [5].

Herbal dentifrices containing *Aloe vera,* have antimicrobial potential on several oral microorganisms, such as *Staphylococcus aureus, Streptococcus mutants, Streptococcus sanguis* and *Candida albicans* [4, 6]. Different studies discussed the benefits of *Aloe vera* tooth gel in oral infection and can be safely recommended as an alternative to fluoridated dentifrices in terms of antimicrobial efficacy [5].

The present study was designed for extraction of *Aloe vera* gel; development and optimization of tooth gel formulation using *Aloe vera* gel extract. The formulation was evaluated for smoothness, spreadability and viscosity. The disc diffusion method was used to investigate the *in vitro* antimicrobial activity of formulated tooth gel against the bacterial strain *Staphylococcus aureus*.

MATERIALS AND METHODS

Plant materials

The fresh leaves of the *Aloe Barbadensis* Millar plant were collected from the medicinal plant garden, Bengal School of Technology, Hooghly, West Bengal, an eastern region in India. Medicinal plant gardens are primarily focused on the conservation, cultivation, research and educational activities related to authenticated herbal plant species known for medicinal purposes.

Chemicals

Carbopol 940 was purchased from Loba Chem Pvt Ltd, Mumbai, India and Carboxy Methyl Cellulose (CMC) was purchased from Simson Chem, Mumbai, India. Sodium Lauryl Sulfate (SLS) and Polyethylene Glycol 400 (PEG 400) were purchased from Loba Chem Pvt Ltd, Mumbai, India. Sodium benzoate was purchased from Renkem Laboratory Reagent, Gujarat, India and sodium saccharin was purchased from Qualikems Laboratory Reagents, Delhi, India. Acetone, chloroform, and methanol were purchased from Loba Chem Pvt Ltd, Mumbai, India. All the chemicals used, including the solvents, were of analytical grade.

Extraction of *Aloe vera* gel

A few *Aloe vera* leaves were broken off of an *Aloe Barbadensis* Millar plant and properly washed in water. Using a sharp knife, the top rind was slowly peeled off while carefully avoiding the vascular bundles in the yellow layer that was just below the green rind. The bottom rind was similarly peeled to remove the substantial amount of mucilage that was still attached and the gel was scooped out with a spoon. 50 ml of crude clear *Aloe vera* gel extract was consistently blended by using a magnetic stirrer for 5 min at 1000 Rotations Per Minute (RPM) and plant extract was filtered through a Whatman filter paper. At last, it was transferred into a sterilized, clean glass jar and stored in the refrigerator [7, 8].

Extraction of chlorophyll from *Aloe vera* leaf

After collecting the *Aloe vera* gel, the rinds of the *Aloe vera* leaves were kept in a beaker. The leaf rinds were macerated with 10 ml of 80% acetone using a pestle and mortar. The extract was centrifuged (Laboratory Centrifuge, Remi Lab World, Mumbai, India) at 3000 RPM for 10 min. Finally, the supernatant of the green pigment of chlorophyll was transferred into a beaker [6].

Optimization of formulation using design of experiment (DoE)

Formulation development is a very complex process that sometimes implicates taking decisions about parameters or variables to obtain the best results in a high variability or uncertainty context. Therefore, robust optimization tools can be very useful for obtaining quality formulations. Optimization studies for the given procedure were performed by using 3^2 full factorial design (Design Expert Software Trial version13.0.5), and 9 runs were generated [7]. Two independent factors (variables), carbopol 940 and CMC were selected and evaluated at three levels, i.e., higher level (+1), medium level (0), and lower level (−1). Response surface design was employed to study the effects of independent variables on responses such as viscosity, spreadability and smoothness. All variables for 3^2 full factorial designs are introduced in table 1 and table 2.

Preparation of *Aloe vera* tooth gel

Carbopol 940 and CMC were used in the formulation of tooth gel. Carbopol 940 was added slowly into the water and stirred continuously. Once the carbopol 940 was fully dispersed, CMC was added to the mixture and stirred continuously to prevent clumping. The required quantity of sodium benzoate, PEG 400, and chlorophyll extract were added. Then the required quantity of *Aloe vera* gel extract was added to the above mixture. The herbal mixture was stirred continuously with the required volume of DD water until it becomes homogeneous [9]. Triethanolamine was added dropwise to the formulation for adjustment of the required pH and to obtain gel in the required consistency. Table 3 provides the composition of all ingredients used for the preparation of the formulations.

Table 1: Variables for optimization of formulation using DoE

Independent factor(s)		Response variable (s)	
X_1	Carbopol 940	Y_1	Viscosity
X_2	CMC	Y_2	Spreadability
	-	Y_3	Smoothness

Table 2: 3^2 full factorial design for formulation of *Aloe vera* tooth gel

Formulation	Carbopol 940 (X_1)	CMC (X_2)
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Table 3: Ingredients for *Aloe vera* tooth gel formulation

Ingredient (s)	Formulation batch code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 940 (g)	1	1	1	1.5	1.5	1.5	2	2	2
CMC (g)	1	1.5	2	1	1.5	2	1	1.5	2
Sodium Benzoate (0.05%) (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PEG-400 (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
SLS (g)	1	1	1	1	1	1	1	1	1
Sodium saccharin (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aloe vera (ml)	15								
Chlorophyll	q. s.								
Triethanolamine (ml)	q. s.								
Double Distilled (DD) water (ml)	q. s.								
Total (g)	30								

Evaluation of prepared tooth gel

Transparency

The 10 ml test tube was filled with approximately 5 ml of the prepared gel and its transparency was inspected visually.

pH determination

The pH of the gel formulation was determined using a pH meter (Digital Instruments Corp., Gujarat, India). In this procedure, 1 g of gel was dissolved in 100 ml of deionized water. The electrode was calibrated before use with a standard buffer solution at 4.0, 7.0 and 9.0. The pH readings were taken three times and the average values were calculated [9].

Smoothness

The smoothness of the gel formulation was tested by rubbing between the fingers and observed whether the gel was smooth, clumped, homogenous or rough.

Viscosity

The viscosity of prepared tooth gel was determined using a viscometer (Brookfield viscometer, Brookfield Corp., Canada) [9, 10].

Spreadability

2 g prepared gel was applied to the ground slide (Spreadability Apparatus, Excel Electro Industries and Leasing Pvt., India) under investigation. The prepared gel was placed between this glass slide and another slide for 5 min to expel air and form a uniform film of the gel between the slides. The excess of the gel was scrapped off from the edges. The top plate was then subjected to a pull of 80g with the help of string attached to the hook and the time (second) required by the top slide to cover a distance of 7.5 cm was noted. Better spreadability was indicated by a brief interval [10].

$$S = M. L/T$$

Where,

S= Spreadability

M= Weight in the pan (tied with the upper slide)

L= Length moved by the glass slide

T= Time in seconds needed to separate the top slide from the bottom slide.

Antimicrobial study

The *in vitro* antimicrobial study of formulated tooth gel was performed by disc diffusion method in a triplicate manner by using Muller Hinton agar medium against a pathogenic bacterial strain *Staphylococcus aureus* (*S. aureus*, ML-267). *S. aureus* was initially cultured in nutrient broth and incubated at 37 °C for 24 h and cultured cells tended to multiply in the Muller Hinton agar plates. The formulated tooth gel-containing discs were placed over the bacterial plates and incubated at 37 °C for 24 h, comparing sodium benzoate as the positive control. The diameter of Zone of Inhibition (ZOI) was measured and the results were interpreted [11, 12].

Thin layer chromatography (TLC) of *Aloe vera*

The experiment was conducted on a sheet of aluminum foil, which was coated with a thin layer of adsorbent material, silica gel (Sv Scientific Pvt. Ltd., India). About 10 µl of extracts were gradually applied onto the plate using a micropipette, and the plate was air dried. Solvent system of chloroform: methanol (12:2) was used as mobile phase and on completion of separation, the chromatograms of *Aloe vera* gel extract and formulations were reported using standard procedures (fig. 5) [13].

RESULTS AND DISCUSSION

Any interactions between excipients and phytoconstituents were studied using Attenuated Total Reflectance (ATR, Bruker Corp., USA). In the IR spectrum of carbopol 940, peaks were observed at 2930.88 cm^{-1} and 2860.32 cm^{-1} which were associated with alkane C-H stretching (fig. 1). Three peaks were observed at 2315 cm^{-1}, 2228 cm^{-1} and 2168 cm^{-1} due to alkyne C≡C stretching. Another two peaks were observed at 1693 cm^{-1} and 1678 cm^{-1} were associated with alkene C=C stretching and acid C=O stretching. Two peaks were observed at 1452 cm^{-1}, 1418 cm^{-1} due to the presence of aromatic C=C stretching. Other peaks were observed at 1226 cm^{-1}, 1116 cm^{-1}, and 1041 cm^{-1} were characteristic of alcohol C-O stretching [14].

For the sample of CMC, the presence of a strong maximum at 1509 cm^{-1} and 1693 cm^{-1} respectively, indicates the presence of carboxyl group–COO–. The broad absorption band at 3544–3584 cm^{-1} for both polymers represents the stretching vibrations of the –OH group (fig. 2). The C–H stretching peaks are observed at 2318 cm–1 and 2379 cm^{-1}. The bands at ~1693 cm^{-1} can be assigned to –CH2 scissoring vibrations as well as to the symmetric stretching vibrations of the carboxyl group in the form of salt (–COO–). The IR peaks maximum appeared at 1509 and ~933 cm^{-1} indicating the presence of –OH bending and C–O–C stretching vibrations respectively [15].

The IR spectrum of *Aloe vera* gel extract consists of a single peak at 3363 cm^{-1} due to the presence of N-H stretching. Some peaks were observed at 2295 cm^{-1}, 2167 cm^{-1}, and 2105 cm^{-1} were associated with C≡C stretching. The absorption band at 1644 cm^{-1} was characteristic of C=O stretching which indicates the presence of carbonyl groups (fig. 3). The absorption band at 970-1250 cm^{-1} corresponds to the stretching vibrations of C-O groups of esters and phenols. A single peak at 707 cm^{-1} was recorded due to the presence of C-H bending [16].

The IR spectrum of formulated *Aloe vera* tooth gel containing carbopol 940 and CMC consists of a single peak of 3732 cm^{-1} which represents the stretching vibrations of the –OH group for containing CMC. Peaks were observed at 2919 cm^{-1} and 2852 cm^{-1} which were associated with alkane C-H stretching due to the presence of carbopol 940. CMC gives the peaks of C–H stretching at 2317 cm^{-1} and 2349 cm^{-1} (fig. 4). Another peak observed at 1704 cm^{-1} was associated with alkene C=C stretching and acid C=O stretching. The presence of an *Aloe vera* absorption band at 1644 cm^{-1} was characteristic of C=O stretching which indicates the presence of carbonyl groups. Another peak was observed at 1437 cm^{-1} due to the presence of aromatic C=C stretching. Other peaks observed at 1214 cm^{-1}, and 1247 cm^{-1} were characteristic of alcohol C-O stretching. A single peak at 708 cm^{-1} was recorded due to the presence of C-H bending. Another peak of 1041 cm^{-1} was characteristic of alcohol C-O stretching [16].

After performing the ATR study of *Aloe vera* gel, excipients and formulation; the study report revealed that there were no major changes in the stretching or bending of major functional groups of *Aloe vera* with all excipients. So, incompatibility between *Aloe vera* and excipients was not observed. Another study confirms that *Aloe vera* gel extract and excipients are compatible [16].

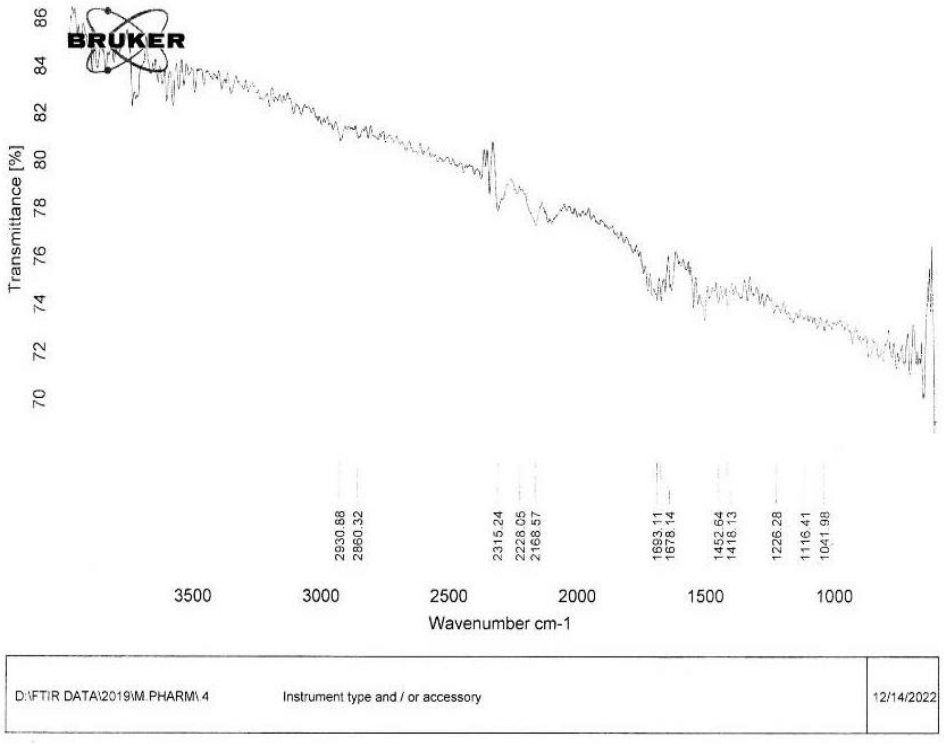

Fig. 1: IR spectrum of carbopol 940

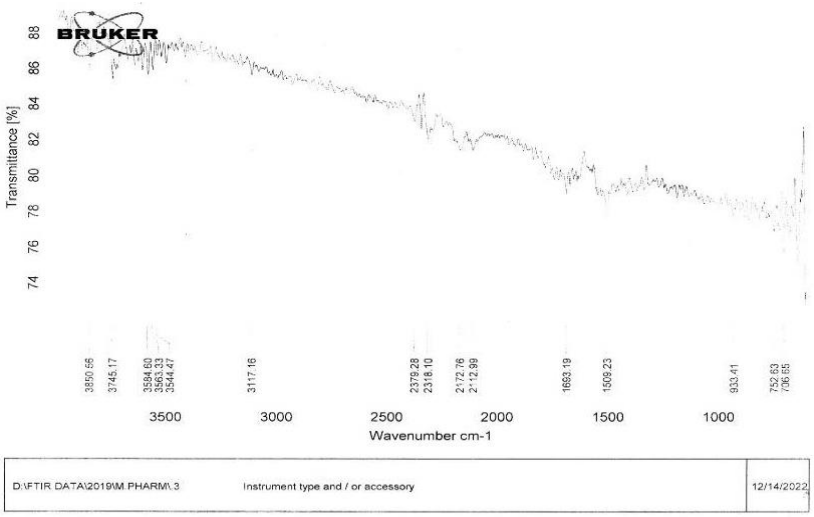

Fig. 2: IR spectrum of sodium CMC

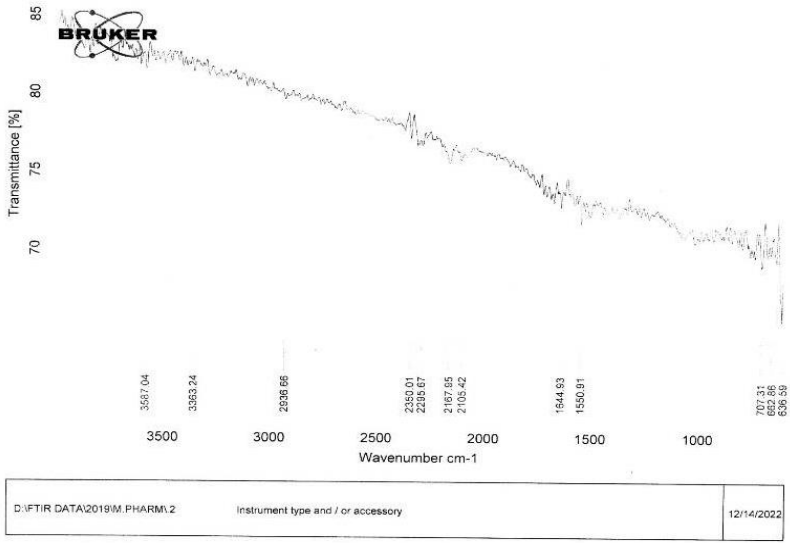

Fig. 3: IR spectrum of *Aloe vera* gel extract

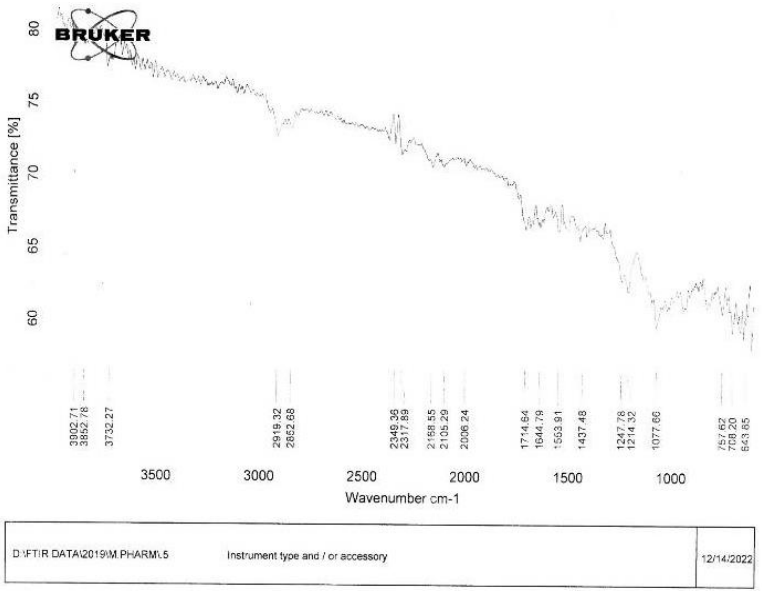

Fig. 4: IR spectrum of tooth gel formulation

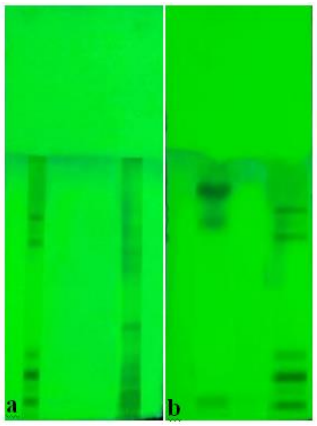

Fig. 5: Thin layer chromatography of a. *Aloe vera* gel b. tooth gel formulation

Thin layer chromatography of methanolic extract of *Aloe vera* gel and prepared tooth gel was carried out (fig. 5). The chromatograms for a methanolic extract of *Aloe vera* gel and tooth gel formulation are subjected to preliminary phytochemical screening (eg. alkaloids, phenolic compounds, glycosides, amino acids, etc.). Another study indicated the presence of phytochemicals in *Aloe vera* gel extract [17, 18].

The developed and formulated A*loe vera* tooth gel was evaluated. The color, transparency, pH, spreadability and viscosity of prepared tooth gel are shown in table 4. Each value in the table was obtained by calculating the average of three experiments (n=3, n i.e. number of experiments) and data are presented as mean±SD. Viscosity and spreadability of the optimized formulation (F4) were found to be 4.9 pa. S and 7 cm/sec respectively. Previously, A*loe vera* tooth gel was evaluated and similar results were observed [5]. The extracted chlorophyll was added to the formulation as a coloring agent [19]. The formulated A*loe vera* tooth gel was found to be yellowish-green in color, translucent in appearance [5].

Table 4: Evaluation of prepared tooth gel, data given in mean±SD (n=3)

Formulation	Color	Transparency	pH	Spreadability (cm/sec)	Viscosity (Pa. S)
F1	Yellowish Green	Slightly transparent	7.5±0.4	16±1	4.033±0.152
F2	Yellowish Green	Slightly transparent	8±1	19±2	3.333±0.208
F3	Yellowish Green	Slightly transparent	8.18±0.99	9.5±2.645	4.366±0.251
F4	Yellowish Green	Translucent	7.9±0.854	7±1	4.9±0.2
F5	Yellowish Green	Translucent	8.11±0.79	3.9±0.2	7.6±0.1
F6	Yellowish Green	Translucent	8.2±1.058	1.2±0.3	10.633±0.568
F7	Yellowish Green	Translucent	7.81±0.578	0.6±0.3	13±1
F8	Yellowish Green	Translucent	8.22±1.07	0.39±0.215	15.266±1.16
F9	Yellowish Green	Translucent	8.13±0.21	0.14±0.036	17.8±0.72

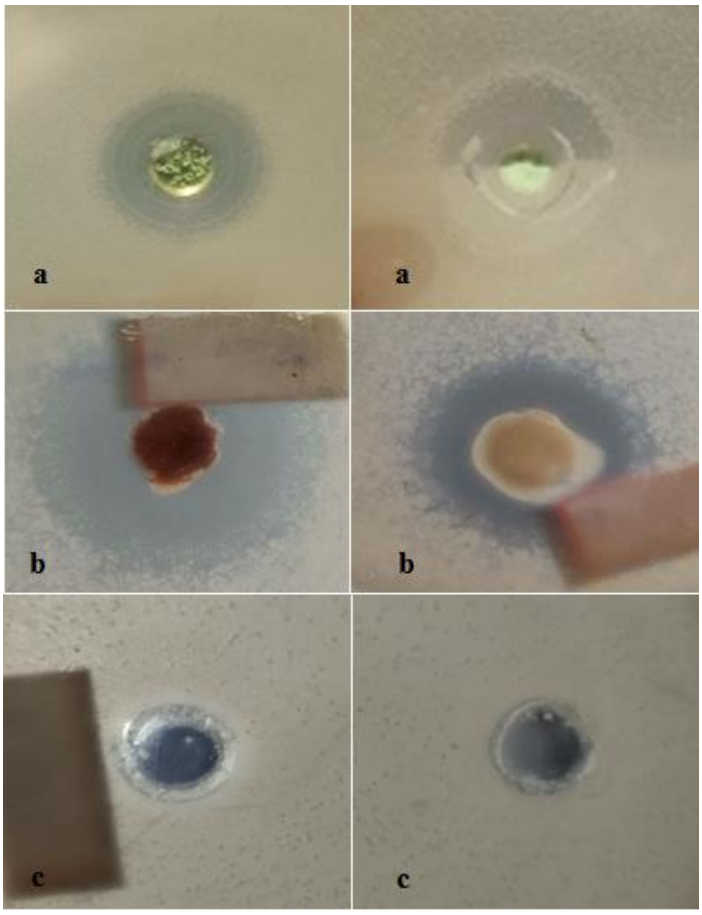

Fig. 6: Antimicrobial study a. prepared tooth gel b. market product c. control

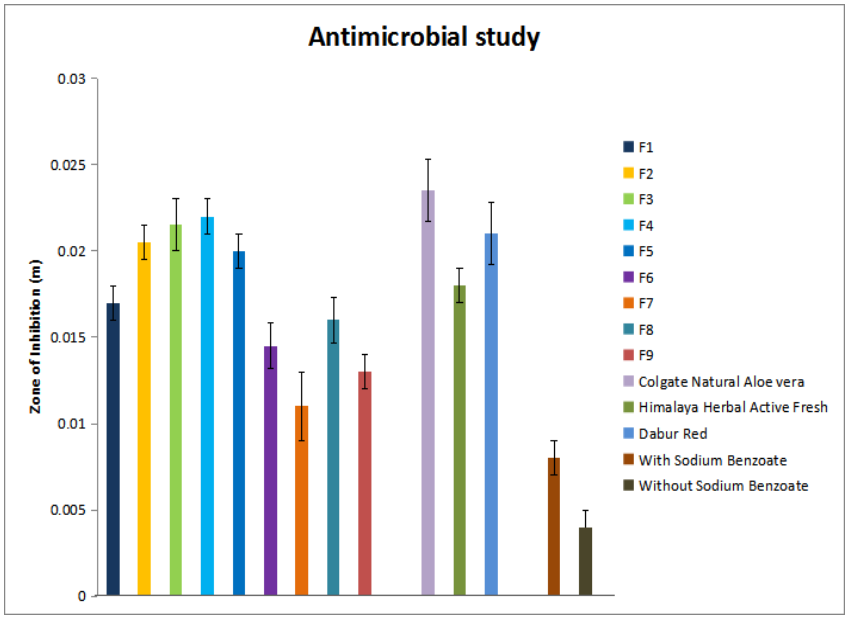

Fig. 7: Zone of Inhibition (ZOI) of formulation, market product and control. Data given in mean±SD (n=3)

The formulation F4 showed a notable activity with ZOI of 0.022 m (fig. 6) and this formulation was considered to be optimized formulation. Therefore, it is concluded that the prepared tooth gel has the potential to exhibit antimicrobial activity against *Staphylococcus aureus* (fig. 7). Similar results were observed by other researchers in a study on antimicrobial activity [17, 20]. Similarly, market products (Colgate Natural Extract A*loe vera*, Himalaya Herbal Active Fresh, Dabur Red) showed antimicrobial activity with ZOI of 0.0235m, 0.018m, and 0.021m respectively. Therefore, it can be concluded that *aloe vera* tooth gel can be used effectively [21]. Studies suggest that *Aloe vera* gel extract is appropriate for treating gingivitis and oral infection, as it inhibits the formation of plaque and the growth of bacteria [22, 23].

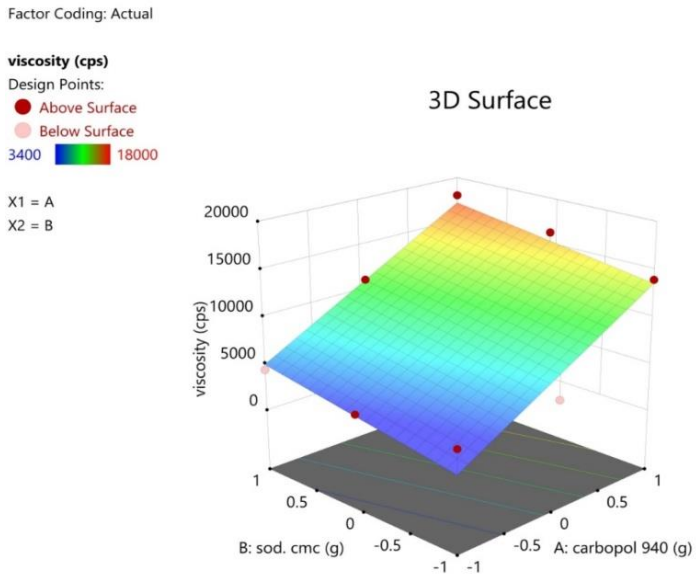

Fig. 8: Response surface curve

Response surface design was employed to investigate the effect of independent variables on response variable. Optimization of herbal tooth gel with respect to the viscosity as a response variable was designed. A 3D response curve was plotted against the CMC, carbopol 940 (independent variables) vs viscosity (response variable). This response curve denoted that the amount of the CMC and the amount of the carbopol 940 was directly proportional to the viscosity of the herbal tooth gel i.e. the amount of CMC and carbopol was increased in the formulation, the viscosity of tooth gel was also increased (fig. 8). Response surface methodology has been reported to be an effective tool for the optimization of a process when the independent variables have a combined effect on the desired response [24, 25].

CONCLUSION

The present research work concluded that the herbal tooth gel was developed and the *Aloe vera* tooth gel was well formulated. The tooth gel was prepared by using carbopol 940 and CMC. The

formulated herbal tooth gel exhibited antimicrobial activity against the bacterial strain *Staphylococcus aureus*. The results from this research evidently noted that the natural plant *Aloe vera* use may be a new approach for tooth gel formulation and good scope in future dental research in natural remedies. Further research related to development, preparation and evaluation of *Aloe vera* tooth gel should be carried out.

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AUTHORS CONTRIBUTIONS

Abu Shoeb performed the experimental work, wrote the manuscript, and helped in drafting and revising the manuscript. Anandamoy Rudra supervised the work and interpreted the results. All authors have made substantial contributions and approved the final manuscript.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this research work.

REFERENCES

1. Babaji P, Melkundi M, Devanna R, S SB, Chaurasia VR, V GP. *In vitro* comparative evaluation of different storage media (hank's balanced salt solution, propolis, Aloe vera, and pomegranate juice) for preservation of avulsed tooth. Eur J Dent. 2017;11(1):71-5. doi: 10.4103/ejd.ejd_101_16, PMID 28435369.
2. Prashar D, Jasra K. Pharmacognostic, phytochemical and therapeutic overview of three allied herbs used in dentistry. Asian J Res Pharm Sci. 2021;11(2):121-5. doi: 10.52711/2231-5659.2021-11-2-5.
3. Kathuria N, Gupta N, Manisha PR. Biologic effects of *Aloe vera* gel. Internet J Microbiol. 2010;9(2):1-6, doi: 10.5580/c3f.
4. Lefsih K, Iboukhoulef L, Petit E, Benouatas H, Pierre G, Delattre C. Anti-inflammatory and antioxidant effect of a d-galactose-rich polysaccharide extracted from aloe vera leaves. ACBC. 2018;1(1):18-26. doi: 10.33513/ACBC/1801-03.
5. Silva TM, Fonseca BM, Sales ALLS, Holleben P, Valera MC, AraUjo MA. Effects of fluoride and Aloe vera tooh gel in artificial white spot lesions *in vitro*. RGO Rev Gauch Odontol. 2016 Jan;64(1):56-61. doi: 10.1590/1981-86372016000100008295 6.
6. Rasala T, Dani S, Waikar S, Ittadwar A. Formulation and evaluation of aloe vera gel and film from fresh pulp of the leaves of aloe barbadensis. AJPHR 2018;6(11):1-9. doi: 10.46624/ajphr.2018.v6.i11.001.
7. Hu Y, Xu J, Hu Q. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. J Agric Food Chem. 2003 Dec 17;51(26):7788-91. doi: 10.1021/jf034255i, PMID 14664546.
8. Ferruzzi MG, Blakeslee J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutr Res. 2007 Jan 1;27(1):1-12. doi: 10.1016/j.nutres.2006.12.003.
9. Al-Nima AM. Preparation and evaluation of topical gel containing *Aloe vera* exudate. IJP. 2022 Jan 10;18(2):39-52. doi: 10.33899/iphr.2022.170396.
10. Sarkar U, Raha A, Mukherjee P, Paul M, Bagchi A. Development and evaluation of metronidazole containing topical gel using different gelling agents. Asian J Pharm Pharmacol. 2018;4(6):785-9. doi: 10.31024/ajpp.2018.4.6.10.
11. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 1949;24(1):1-15. doi: 10.1104/pp.24.1.1, PMID 16654194.
12. Giri MA, Bhalke RD. Formulation and evaluation of topical anti-inflammatory herbal gel. Asian J Pharm Clin Res. 2019;12(7):252-5. doi: 10.22159/ajpcr.2019.v12i7.33859.
13. Ramirez Duron R, Ceniceros Almaguer L, Cavazos Rocha NC, Silva Flores PG, De Torres NW. Comparison of high-performance liquid chromatographic and thin-layer chromatographic methods for determination of aloin in herbal products containing *Aloe vera*. J AOAC Int. 2008 Nov 1;91(6):1265-70. doi: 10.1093/jaoac/91.6.1265, PMID 19202785.
14. VLG, VDY, RPD, PBC, SDJ. Effect of carbopol 934 and 940 on fluconazole release from topical gel formulation: a factorial approach. JCPR. 2012;2(2):487-93. doi: 10.33786/JCPR.2012.v02i02.004.
15. Ridwan S, Hartati R, Pamudji JS. Development and evaluation of cream preparation containing phytosome from *amla* fruit extract (*Phyllanthus emblica* L.). Int J App Pharm. 2023;15(4):91-8. doi: 10.22159/ijap.2023v15i4.48116, doi: 10.22159/ijap.2023v15i4.48116.
16. Abbasi MSA, Tahir MA, Meer S. FTIR spectroscopic study of *Aloe vera barbadensis* mill buds. AJOCS. 2020 Apr 27;7(4):1-6. doi: 10.9734/ajocs/2020/v7i419026.
17. Roshanak S, Behbahani BA, Shahidi F, Yazdi FT, Vasile AR, Norouzi N. Evaluation of chlorophyll content, antioxidant activity and antimicrobial effect of *dandelion* leaves extract, Iran Food Sci Technol Res J. 2021;17(3):63-72. doi: 10.22067/ifstrj.v17i3.82421.
18. Raad B. Phytochemical screening and biological activities of *Aloe vera* (L.) Burm. F. Pure Appl Biol. 2021 Jun;10(1):360-7. doi: 10.19045/bspab.2021.100039.
19. Ebrahimi P, Shokramraji Z, Tavakkoli S, Mihaylova D, Lante A. Chlorophylls as natural bioactive compounds existing in food by-products: a critical review. Plants (Basel). 2023;12(7):1533. doi: 10.3390/plants12071533, PMID 37050159.
20. Padole NN. Synthesis of silver nanoparticles for antibacterial activity against staphylococcus aureus and escherichia coli. Asian J Pharm Res Dev 2007;10(2):29-36. doi: 10.22270/ajprd.v10i2.1101.
21. Athiban PP, Borthakur BJ, Ganesan S, Swathika B. Evaluation of antimicrobial efficacy of Aloe vera and its effectiveness in decontaminating gutta percha cones. J Conserv Dent. 2012 Jul;15(3):246-8. doi: 10.4103/0972-0707.97949, PMID 22876011.
22. Kriplani R, Thosar N, Baliga MS, Kulkarni P, Shah N, Yeluri R. Comparative evaluation of antimicrobial efficacy of various root canal filling materials along with aloevera used in primary teeth: a microbiological study. J Clin Pediatr Dent. 2013;37(3):257-62. doi: 10.17796/jcpd.37.3.j62u53q2300484x5. PMID 23855169.
23. Jain S, Rathod N, Nagi R, Sur J, Laheji A, Gupta N. Antibacterial effect of aloe vera gel against oral pathogens: an *in vitro* study. J Clin Diagn Res. 2016 Nov;10(11):ZC41-4. doi: 10.7860/JCDR/2016/21450.8890, PMID 28050502.
24. Koocheki A, Taherian AR, Razavi SMA, Bostan A. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from *Lepidium perfoliatum* seeds. Food Hydrocoll. 2009 Dec;23(8):2369-79, doi: 10.1016/j.foodhyd.2009.06.014.
25. Murti VSR, Philip PK. A comparative analysis of machining characteristics in ultrasonic assisted EDM by the response surface methodology. Int J Prod Res. 1987 Feb 1;25(2):259-72. doi: 10.1080/00207548708919838.