International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com
ISSN (Online): 2581-5782

Polyherbal Toothgel Containing Extracts of Punica Granatum Peels and Psidium Guajava Leaves Using Chitosan and Carrageenan Gel Bases: A Comparative Study

Saranya M[1], Sarath chandran C[2], Vishnu A. S[3]

[1]*M. Pharm. Student, Department of Pharmaceutics, Academy of pharmaceutical Sciences, Kannur, India*
[2]*Assistant Professor, Department of Pharmaceutics, Academy of pharmaceutical Sciences, Kannur, India*
[3]*Lecturer, Department of Pharmaceutics, Academy of pharmaceutical Sciences, Kannur, India*

***Abstract*: Due to the awareness of harmful side effects of synthetic ingredients used in commercial toothgels or pastes, people are now more attracted towards the toothgels made of natural ingredients. Therefore, this study was aimed to formulate and evaluate new polyherbal toothgel containing extracts of Punica granatum (Pomegranate) peels and Psidium guajava (Guava) leaves. The formulated toothgels were completely free of synthetic ingredients. The formulations were prepared by using two kinds of natural gel bases-chitosan and carrageenan. All the developed formulations were evaluated by various evaluation parameters such as pH, hard and sharp edged abrasive material, foaming ability, cleaning ability, spreadability, extrudability, viscosity, in vitro antimicrobial activity etc. The antimicrobial activity of the formulated toothgels were tested against Escherichia coli and Candida albicans. The formulated toothgels showed significant inhibition against the test microorganisms. Overall, the formulations developed by using chitosan gel base showed superior quality in comparison with the formulations containing carrageenan gel base.**

Keywords: Polyherbal toothgel, antibacterial activity, *Punica granatum, Psidium guajava*.

I. Introduction

The oral care products that are used by individuals on a daily basis in order to promote oral hygiene are called dentifrices. Among the commonly available dentifrices in the market tooth gels or pastes are most preferred by the global population [1]. The ingredients of a tooth gel include active ingredients, abrasives, whitening agents, humectants, gelling agents, colouring agents, flavouring agents and preservatives.

The problem now is the long term use of most of the commercially available tooth gels in the market may cause serious health hazards [2]. In commercial tooth gels, the generally used whitening agents include bleach and peroxides, both of which can act as a mouth and skin irritant in small doses and may cause severe chemical burns in large doses. The commercial tooth gel or paste ingredients may affect the environment adversely. The formaldehyde and Ethylene Diamine Tetra Aceticacid (EDTA), the commonly used preservatives in tooth gels may cause environmental pollution3. Oral care products containing antimicrobial agents such as triclosan, cetylpyridinium chloride, amine fluorides etc. are reported to exhibit toxicity and cause staining of the teeth4. Chlorhexidine gluconate (CG), which is considered as the gold standard for clinical efficacy in plaque control is reported with discolouration of mouth and teeth, tartar formation on the teeth, taste problems etc. In addition, CG is reported with serious allergic reactions5. Considering these reasons, the study was selected to overcome the problems associated with synthetic tooth gels by developing a tooth gel formulation which is completely free of synthetic ingredients. The investigation suggested the possibilities to formulate a novel tooth gel containing extracts of *Punica granatum* peel and *Psidium guajava* leaves along with other natural excipients which are safe as well as effective without any side effects.

II. Materials and Methods

A. Collection of plant materials and identification

Fresh fruits of pomegranate, tender guava leaves, aloe vera, beetroot and licorice root were collected from different localities of Kannur and Kasaragod district and identified.

B. Preparation of herbal extracts

The peels of pomegranate were separated manually from the fruits, cut into small pieces and dried under shade (27 ± 2° C). The dried peels were coarsely powdered and 100 g of powdered peels were subjected to extraction by maceration in 500 ml 70 % methanol at 27 ± 2° C for 7 days. The macerates were filtered and the filtrate was dried at a temperature of 27 ± 2° C for 10 days. The dried extract was stored in a freezer (refrigerator) at 4 ± 2° C until further use [2].

The tender leaves of guava was dried under shade (27 ± 2° C) and coarsely powdered using an electric mixer grinder. The powder was defatted with petroleum ether and subjected to soxhlet extraction using 500 ml 70 % methanol. The extract was collected and subjected to solvent evaporation from a china dish. The dried extract was stored in a freezer (refrigerator) at 4 ± 2° C until further use [6].

C. Determination of MIC of combined herbal extract

The MIC of the combined herbal extract was prepared by tube dilution method, by taking the extracts of pomegranate

International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com
ISSN (Online): 2581-5782

peel and guava leaves in 1:1 ratio. A series of concentration of combined herbal extract was prepared in Dimethyl sulfoxide where the concentration ranges between 1000 µg/ml and 62.5 µg/ml. Each test tubes along with positive and negative control was inoculated with one drop of microbial culture and incubated at a temperature of 37 ± 2° C for a period of 48 hours. After incubation, all the test tubes were examined for the growth in the form of turbidity. The results were recorded and the MIC was calculated by comparing all the results with positive and negative control [7].

D. Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard procedures (Table-1) [8].

TABLE I
IDENTIFICATION OF PHYTOCHEMICAL CONSTITUENTS

Phytochemical constituents	Methanolic extract of *Punica granatum*	Methanolic extract of *Psidium guajava*
Flavonoids	+	+
Phenolic compounds	+	+
Terpenes	-	+
Tannins	+	+
Glycosides	-	+
Saponins	-	+

+ Presence of phytochemical constituents
- Absence of phytochemical constituents

E. Preparation of other herbal ingredients

Gel of aloe vera

The fresh aloe vera leaves were collected and the gel from aloe vera leaves were directly scraped out. The gel was crushed and used for the formulation of tooth gel.

Powder of licorice root

The dried roots were crushed into fine powder and passed through sieve number #170. The fine powder obtained after sieving is stored in an air tight container for further use.

Powder of eggshell

The poultry eggshells were collected and cleaned in distilled water. Then, the eggshells were kept in a hot water bath at a temperature of 100 ± 2° C for 20 minutes followed by removal of the membrane. The eggshells were air dried and then crushed using a mortar and pestle, and passed through sieve number # 170. The powdered eggshell was stored in an air tight container for further use [9], [10].

Beetroot colour

The beetroot was cut into small pieces and 5g of the cut pieces were placed in a mortar and crushed with a pestle. A mixture of hexane and acetone at 1:1 ratio was added into the mortar and the sample was crushed again. To the mortar, 5 ml of acetone was added again and triturated well. The solvent was collected and filtered through Whatmann filter paper No.1. The filtrate was then transferred into a separating funnel. 50 ml of distilled water was added along with the addition of 50 ml 10 % NaCl solution into the separating funnel. The mixture was shaken vigorously and kept aside for the layers to separate. The upper layer containing coloured pigments were collected separately after the removal of water and NaCl solution. The extract was collected in tubes [11].

F. Preparation of gel bases

4% and 7% was fixed as the concentration for chitosan and carrageenen respectively, on trial and error basis.

Preparation of 4% chitosan gel base

4g chitosan was weighed and soaked in 1% glacial acetic acid solution. It was then kept for 24 hours to get a clear gel of 4% chitosan [12].

Preparation of 7% carrageenan gel base

Required quantity of carrageenan powder was weighed accurately and allowed to swell in sufficient quantity of hot distilled water to get a clear gel of 7% carrageenan [13].

G. Preparation of tooth gel

The formulations were designed as per its composition listed in Table-2 and Table-3. The weighed quantity of eggshell powder and licorice root powder were taken in a mortar and mixed well. Weighed quantity of the extracts of pomegranate peel and guava leaves were mixed thoroughly with a small portion of gel base on an ointment slab. The contents from the mortar was transferred to the ointment slab and blended well with the above mixture. The blended mixture was then transferred from the ointment slab to a clean dry mortar. To this, weighed quantity of aloe vera gel was added followed by the addition of beetroot colour and clove oil. The contents are then triturated well using a pestle and finally transferred into aluminium tube until further use [14].

H. Evaluation of tooth gel formulation

1. Preliminary characteristics

The preliminary characterization of developed as well as marketed sample of tooth gel was done in terms of organoleptic properties and drying tendency [15].

TABLE II
COMPOSITION OF DEVELOPED FORMULATIONS CONTAINING 4% CHITOSAN GEL BASE

Ingredients*	Formulation code								
	CF1	CF2	CF3	CF4	CF5	CF6	CF7	CF8	CF9
Pomegranate peel extract	1	2	3	4	5	9	8	7	6
Guava leaf extract	9	8	7	6	5	1	2	3	4
Eggshell powder	30	30	30	30	30	30	30	30	30
Aloe vera gel	20	20	20	20	20	20	20	20	20
Licorice root powder	1	2	3	1	2	3	1	2	3
Clove oil	1	2	3	1	2	3	1	2	3
Beetroot colour	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1
4% chitosan gel base	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

*All ingredients added in % w/w

International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com
ISSN (Online): 2581-5782

TABLE III
COMPOSITION OF DEVELOPED FORMULATIONS CONTAINING 7% CARRAGEENAN GEL BASE

Ingredients*	Formulation code								
	CGF1	CGF2	CGF3	CGF4	CGF5	CGF6	CGF7	CGF8	CGF9
Pomegranate peel extract	1	2	3	4	5	9	8	7	6
Guava leaf extract	9	8	7	6	5	1	2	3	4
Eggshell powder	30	30	30	30	30	30	30	30	30
Aloe vera gel	20	20	20	20	20	20	20	20	20
Licorice root powder	1	2	3	1	2	3	1	2	3
Clove oil	1	2	3	1	2	3	1	2	3
Beetroot colour	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1
7% carrageenan gel base	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

*All ingredients added in % w/w

2. Physico-chemical characteristics

The evaluation of physicochemical characteristics was done in terms of abrasiveness, pH, viscosity, spreadability and extrudability [15].

3. Performance evaluation

Performance evaluations were made on the basis of foaming ability, cleaning ability and in vitro antimicrobial profile. The developed as well as marketed sample of tooth gel was subjected for performance evaluations.

4. Accelerated stability studies

Accelerated stability studies of all developed formulations were performed as per WHO guidelines 2017 with necessary modifications. The formulated tooth gels were filled into collapsible aluminum tubes and stored at different temperature and humidity conditions, 25°C ± 2°C/60% RH ± 5%, 40°C ±2 °C/75% RH ± 5% for a period of 1 month and observed for change in colour, consistency, pH, viscosity and extrudability [16].

III. RESULTS AND DISCUSSION

A. MIC of the extracts

The MIC for the combined herbal extract was determined against the strains of *E coli* (ATCC 25922) and *C albicans* (ATCC 10231), and the MIC value was found to be 800 µg/ml, which was further confirmed by cup plate method (Fig. 1).

Fig. 1. Cup plate method for combined

Methanolic herbal extracts using *E coli* (ATCC 25922) and *C albicans* (ATCC 10231).

Based on the preliminary evaluation, the formulations containing 4% chitosan gel base was much better as compared to the formulations with 7% carrageenan gel base (Table-4). None of the formulations produced any kind of drying tendency.

TABLE IV
ORGANOLEPTIC PROPERTIES OF THE FORMULATIONS

Formulations	Organoleptic properties*		
	Colour	Consistency	Texture or feel
CF1	Dark green	Gel	Non sticky and smooth
CF2	Light green	Gel	Non sticky and smooth
CF3	Light green	Gel	Non sticky and smooth
CF4	Dark green	Gel	Non sticky and smooth
CF5	Light green	Gel	Non sticky and smooth
CF6	Light green	Gel	Non sticky and smooth
CF7	Dark green	Gel	Non sticky and smooth
CF8	Light green	Gel	Non sticky and smooth
CF9	Light green	Gel	Non sticky and smooth
CGF1	Dark green	Thick gel	Sticky and smooth
CGF2	Light green	Thick gel	Sticky and smooth
CGF3	Light green	Thick gel	Sticky and smooth
CGF4	Dark green	Thick gel	Sticky and smooth
CGF5	Light green	Thick gel	Sticky and smooth
CGF6	Light green	Thick gel	Sticky and smooth
CGF7	Dark green	Thick gel	Sticky and smooth
CGF8	Light green	Thick gel	Sticky and smooth
CGF9	Light green	Thick gel	Sticky and smooth
MS	Green	Gel	Non sticky and smooth

*All samples were taken in triplicate

The pH of all the developed formulations were within the acceptable range [15], [17] and none of the developed formulations showed the presence of any hard and sharp edged abrasive material. There was a significant change in viscosity, spreadability and extrudability between the formulations (Table-5, 6, 7 and 8). Formulations with 7% carrageenan gel base was observed to be having higher viscosity and hence, poor spreadability and extrudability compared to the formulation with 4% chitosan gel base.

TABLE V
ORGANOLEPTIC PROPERTIES OF THE FORMULATIONS

Formulation code	Spindle No	rpm	*Viscosity (cps)	Torque %
CF1	63	20 50 100	12608 12576 12530	94.2 ± 0.10 96.3 ± 0.68 96.5 ± 0.53
CF2	63	20 50 100	11867 11765 11632	95.6 ± 0.42 96.4 ± 0.41 96.8 ± 0.23
CF3	63	20 50 100	11936 11878 11666	96.2 ± 0.18 96.4 ± 0.42 96.8 ± 0.63
CF4	63	20 50 100	12364 12228 12113	94.4 ± 0.62 95.6 ± 0.46 96.6 ± 0.18
CF5	63	20 50 100	12835 12665 12334	94.6 ± 0.21 94.8 ± 0.36 95.6 ± 0.32
CF6	63	20 50 100	11876 11543 11435	95.4 ± 0.81 96.4 ± 0.62 96.8 ± 0.52
CF7	63	20 50 100	11984 11864 11456	95.2 ± 0.65 96.4 ± 0.55 96.6 ± 0.43
CF8	63	20 50 100	12889 12654 11376	92.6 ± 0.55 94.8 ± 0.43 95.2 ± 0.39
CF9	63	20 50 100	12367 12234 12211	94.3 ± 0.16 95.2 ± 0.26 95.8 ± 0.22

* All samples were taken in triplicate

International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com
ISSN (Online): 2581-5782

TABLE VI
VISCOSITY OF THE FORMULATIONS CONTAINING 7% CARRAGEENAN GEL BASE

Formulation code	Spindle No	rpm	*Viscosity (cps)	Torque %
CGF1	63	20	16996	70 ± 0.71
		50	14335	72 ± 0.92
		100	14234	76 ± 0.86
CGF2	63	20	16667	71 ± 0.45
		50	15443	73 ± 0.66
		100	15322	74 ± 0.31
CGF3	63	20	15886	73 ± 0.86
		50	15667	75 ± 0.81
		100	15443	76 ± 0.42
CGF4	63	20	16984	70 ± 0.84
		50	16354	71 ± 0.64
		100	16234	74 ± 0.48
CGF5	63	20	16543	71 ± 0.58
		50	16234	74 ± 0.93
		100	16133	75 ± 0.82
CGF6	63	20	15998	72 ± 0.13
		50	15344	73 ± 0.24
		100	15233	75 ± 0.19
CGF7	63	20	16775	70 ± 0.26
		50	16554	72 ± 0.35
		100	16222	74 ± 0.49
CGF8	63	20	15997	72 ± 0.29
		50	15433	73 ± 0.88
		100	15222	75 ± 0.92
CGF9	63	20	16778	71 ± 0.32
		50	15998	72 ± 0.45
		100	15886	74 ± 0.56

* All samples were taken in triplicate

TABLE VII
SPREADABILITY OF THE FORMULATIONS

Formulation code	Time taken to slide (seconds)	Spreadability*
CF1	4.2	28.57 ± 0.39
CF2	5.1	23.53 ± 0.35
CF3	4.6	26.09 ± 0.32
CF4	4.2	28.57 ± 0.38
CF5	4.7	25.53 ± 0.29
CF6	5.2	23.08 ± 0.23
CF7	5.5	21.82 ± 0.33
CF8	5.1	23.53 ± 0.22
CF9	5.5	21.82 ± 0.31
CGF1	59.2	2.03 ± 0.08
CGF2	56.5	2.12 ± 0.09
CGF3	57.8	2.08 ± 0.05
CGF4	56.6	2.12 ± 0.04
CGF5	52.2	2.29 ± 0.06
CGF6	60.2	1.99 ± 0.02
CGF7	54.2	2.21 ± 0.03
CGF8	56.5	2.12 ± 0.01
CGF9	52.2	2.29 ± 0.09
MS	4.4	27.27 ± 0.05

*All Samples were taken in triplicate

TABLE VIII
EXTRUDABILITY OF THE FORMULATIONS

Formulation code	Extrudability*
CF1	++++
CF2	++++
CF3	++++
CF4	++++
CF5	++++
CF6	++++
CF7	++++
CF8	++++
CF9	++++
CGF1	+
CGF2	+
CGF3	+
CGF4	+
CGF5	+
CGF6	+
CGF7	+
CGF8	+
CGF9	+
MS	++++

* ++++ Excellent, +++ Good, ++ Satisfactory, + Poor
* All samples were taken in triplicate

Irrespective of the gelling agent used, the formulations exhibited different foaming (Table-9) and cleaning ability (Fig. 2, 3 and 4).

TABLE IX
FOAMING ABILITY OF THE FORMULATIONS

Formulation Code	Volume of water (V1)	Volume of foam with water (V2)	Foaming ability* (V2-V1)
CF1	30	34	4 ± 1.00
CF2	30	39	9 ± 1.52
CF3	30	40	10 ± 1.00
CF4	30	32	2 ± 1.20
CF5	30	39	9 ± 0.50
CF6	30	41	11 ± 0.62
CF7	30	33	3 ± 1.23
CF8	30	39	9 ± 1.00
CF9	30	41	11 ± 1.34
CGF1	30	32	2 ± 0.56
CGF2	30	39	9± 0.88
CGF3	30	40	10 ± 0.76
CGF4	30	33	3 ± 0.77
CGF5	30	39	9 ± 1.33
CGF6	30	40	10 ± 1.26
CGF7	30	32	2 ± 0.50
CGF8	30	34	9 ± 0.46
CGF9	30	40	10 ± 1.50
MS	30	40	10 ± 0.56

*All samples were taken in triplicate

Fig. 2. Coloured egg for evaluation

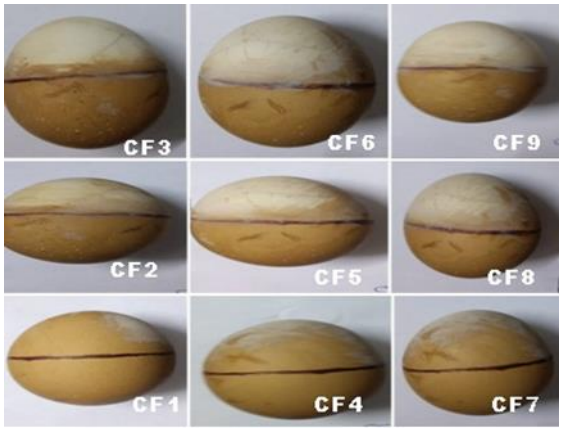

Fig. 3. Cleaning ability of formulations containing 4% chitosan gel base

The *in vitro* antimicrobial evaluation of developed formulations indicated that all the developed formulas with 4% chitosan and 7% carrageenan gel base showed activity against both *E coli* (ATCC 25922) and *C albicans* (ATCC 10231). Among the formulations, CF9 produced maximum zone of inhibition against both of the test microorganisms (Table-10, Fig. 5)

International Journal of Research in Engineering, Science and Management
Volume-1, Issue-9, September-2018
www.ijresm.com
ISSN (Online): 2581-5782

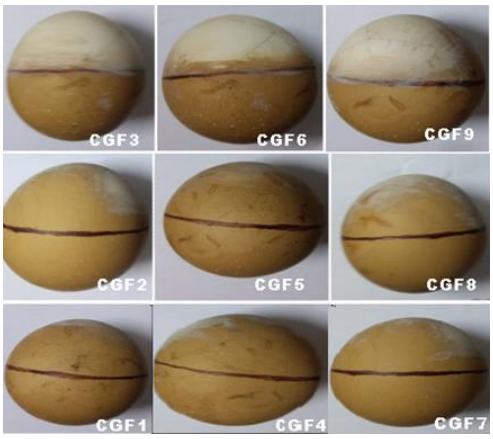

Fig. 4. Cleaning ability of formulations containing 7% carrageenan gel base

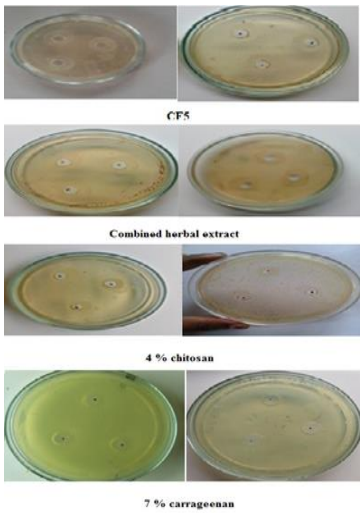

Fig. 5. *In vitro* antimicrobial activity of the formulations

TABLE X
IN VITRO ANTIMICROBIAL TEST ON TOOTH GEL FORMULATIONS

Formulation code	*Zone of inhibition for *E coli* (mm)	*Zone of inhibition for *C albicans* (mm)
CF1	4.5 ± 0.10	5 ± 0.10
CF2	5 ±0.15	5.5 ± 0.10
CF3	6 ± 0.23	6.5 ± 0.10
CF4	6 ± 0.22	7.5 ± 0.10
CF5	9 ± 0.26	9 ± 0.10
CF6	5 ± 0.36	4 ± 0.10
CF7	4.5 ± 0.24	4.5 ± 0.10
CF8	4 ± 0.12	6 ± 0.10
CF9	8.5 ± 0.26	8.5 ± 0.10
CGF1	3 ± 0.18	3.5 ± 0.10
CGF2	3.5 ± 0.26	4 ± 0.10
CGF3	4.5 ± 0.12	5 ± 0.10
CGF4	6.5 ± 0.26	7 ± 0.10
CGF5	8 ± 0.14	8.5 ± 0.10
CGF6	3.5 ± 0.18	4.5 ± 0.10
CGF7	3 ± 0.24	3 ± 0.10
CGF8	4.5 ± 0.12	5.5 ± 0.10
CGF9	7 ± 0.16	8.5 ± 0.10
MS	3.5 ± 0.10	4 ± 0.10
4 % chitosan gel base	4 ± 0.12	3.5 ± 0.16
7 % carrageenan gel base	-	-
Combined herbal extract	6 ± 0.12	6.5 ± 0.24

*All samples were taken in triplicate

Based on the results of one month accelerated stability studies on the developed formulations, the formulations CF1-CF9 with 4% chitosan gel base exhibited the properties that are mandatory for a good tooth gel (Table-11, 12).

TABLE XI
ACCELERATED STABILITY STUDIES OF FORMULATIONS WITH 4% CHITOSAN GEL BASE

Formulation code	*Evaluation parameters (After one month)				
	Colour	Consistency	pH	Viscosity	Extrudability
CF1	Dark green	Gel	6.6 ± 0.28	No change	++++
CF2	Light green	Gel	6.5 ± 0.30	No change	++++
CF3	Light green	Gel	6.6 ± 0.42	No change	++++
CF4	Dark green	Gel	6.6 ± 0.22	No change	++++
CF5	Light green	Gel	6.8 ± 0.36	No change	++++
CF6	Light green	Gel	6.7 ± 0.18	No change	++++
CF7	Dark green	Gel	6.5 ± 0.46	No change	++++
CF8	Light green	Gel	6.4 ± 0.24	No change	++++
CF9	Light green	Gel	6.5 ± 0.82	No change	++++

*All samples were taken in triplicate

TABLE XII
ACCELERATED STABILITY STUDIES OF FORMULATIONS WITH 7 % CARRAGEENAN GEL BASE

Formulation code	*Evaluation parameters (After one month)				
	Colour	Consistency	pH	Viscosity	Extrudability
CGF1	Dark green	Hard gel	6.9 ± 0.12	Increased	+
CGF2	Light green	Hard gel	7.1 ± 0.22	Increased	+
CGF3	Light green	Hard gel	7.2 ± 0.43	Increased	+
CGF4	Dark green	Hard gel	7.4 ± 0.32	Increased	+
CGF5	Light green	Hard gel	7.2 ± 0.21	Increased	+
CGF6	Light green	Hard gel	7.1 ± 0.14	Increased	+
CGF7	Dark green	Hard gel	6.9 ± 0.16	Increased	+
CGF8	Light green	Hard gel	7.1 ± 0.22	Increased	+
CGF9	Light green	Hard gel	7.1 ± 0.12	Increased	+

*All samples were taken in triplicate

IV. CONCLUSION AND RECOMMENDATION

From the evaluation studies performed over the developed tooth gel formulations, it was found out that the formulations containing 4 % chitosan gel base had superiority in almost all the characteristics that are mandatory for a good tooth gel. Hence, the formulations CF1-CF9 containing 4% chitosan gel base was found to be better as compared to the formulations CGF1-CGF9 that contained 7% carrageenan gel base. The formulated tooth gels may be safer compared to fully synthetic tooth pastes and further studies are warrented to prove safety and efficacy of the formulated tooth gels.

REFERENCES

[1] Mangilal T, Ravikumar M. Preparation and evaluation of herbal tooth paste and comparison with commercial herbal tooth pastes; an invitro study. Int. J. Ayur. & Herb. Med. 2016;6 (3):2251-2266.
[2] Mahendran S, Muhammad ZA. Formulation, evaluation and antimicrobial properties of polyherbal tooth paste. Int J Curr Pharm Res. 2016; 8 (3):105-107.
[3] Abhay S, Dinnimath BM, Hullatti KK. Formulation and spectral analysis of new polyherbal tooth paste. J. Drug Del. & Ther. 2014; 4(6):68-74.
[4] Abhay S, Dinnimath BM. Formulation and evaluation of new polyherbal tooth paste for oral care. Ind. J. Health Sci. 20158(1):24-27.
[5] Vishnu PSG, Reema L. Characteristics, uses and side effects of Chlorhexidine- A review. J. Dent & Med. Sci. 2016; 15(6):57-59.
[6] Mahendran S, Noor JSA. Formulation, evaluation and antibacterial properties of novel polyherbal tooth paste for oral care. Int. J. Pharm. & Clin. Res. 2016; 8 (8):1155-1158.
[7] Dubey RC, Maheshwari DK. Practical Microbiology. 2nd edition. Career publications. 2004; 155.
[8] Prasant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: a review. Int. Pharmaceutica. Sci. 2011; 1(1):98-106.
[9] Gaurav BD, Navin KK. Formulation and evaluation of tooth paste by using eggshells. World. J. Pharm. Res. 2017; 6(2):534-543.
[10] Uduma EO, Chidozie SO, Sunday OA, Onyinie JU, Michael UA. Eggshell powder as a potent direct compression excipient in tablet formulation. W. African J. Pharm. 2017; 28 (1):107-118.
[11] Jeyanthi RL, Sharmila S, Merina PD, Candace S. Extraction and purification of carotenoids from vegetables. J.Chem.Pharm.Res. 2014; 6 (4):594-598.
[12] Navneet B, Kamlesh K, Dhiraj S. Synthesis of chitosan based gel and study of swelling characteristics. Indian. J. Chem. Technol. 2017; 24:560-562.
[13] Rosemary H. Formulation of air freshner gel with carrageenan as gelling agent, lemon oil as fragrance and patchouli oil as binder. Int. J. Chem. Tech. Res. 2017; 10 (4):207-212.
[14] Poucher's perfumes, cosmetics and soaps. 10thedition. Springer International Edition. 217-253.
[15] Kandarp D, Lata P, Pragna KS. Development and evaluation of antibacterial herbal tooth paste containing Eugenia caryophillus, Acacia nilotica and Mimusopselengi. Int. J. Chem. & Pharm. Sci. 2014; 2 (3):666-673.
[16] Stability testing of active pharmaceutical ingredients and finished products: World Health Organiation; 2017
[17] Mohammed K, Farzin F, Shirin M. Formulation and physicochemical evaluation of toothpaste formulated with Thymus vulgaris essential oil. J HerbMed Pharmacol. 2017; 6 (3):130-135.