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Research Article

# FORMULATION AND EVALUATION OF MEDICATED CHEWING GUM OF EGCG (EPIGALLOCATECHIN GALLATE) ENRICHED EXTRACT OF *CAMELLIA SINENSIS* (GREEN TEA) FOR PERIODONTAL DISEASE

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## ABSTRACT
The aim of this study was the formulation and evaluation of medicated chewing gum of EGCG (epigallocatechin gallate) enriched extract of *camellia sinensis*. The extraction of marketed lipton green tea was done by infusion method and antibacterial activities of extract were done against *P. gingivalis* and *S. mutan* by well diffusion method. Chewing gum was prepared by softening of gum bases and then mixing with other formulation ingredients and optimization of the formulation by screening of different excipients. Performance evaluation was carried out by evaluating hardness, fracturability, adhesiveness, elasticity, cohesiveness, stringiness, chewiness, gumminess, *in vivo* drug release study. Precentage yield of extract in first step and second step were found to be 9.43, 11.65 respectively by infusion method. The EGCG obtained with second step of two step method was found significantly greater. In antibacterial activities, the glucan synthesis by the bacterial *S. mutan* glucosyl transferase was strongly inhibited by (-)-epigallocatechin gallate (EGCG) at concentrations of 50-200ug/ml, the main components of the green tea polyphenols and completely inhibited the growth and adherence of *P. gingivalis* onto the buccal epithelial cells. Optimized formulations MCG-6 showed hardness, adhesiveness, elasticity, gumminess, chewiness, resilience and cohesiveness values are similar to that of the reference (Nicogum). *In vitro* drug released of optimized formulation was found to be 77% within 15 min. The developed formulation of medicated chewing gum can be a better alternative to mouth dissolving and conventional tablet formulation. It may be proved as a promising approach to improve the bioavailability as well as to improve patient compliance.

**Keywords:** Periodontal diseases, Chewing gum, *Camellia sinensis,* (-)-Epigallocatechin gallate, Antibacterial activity.

## 1. INTRODUCTION
In the recent years, chewing gum being a tasty and enjoyable confectionery has been proven its potential as an effective delivery vehicle for pharmaceutical and nutraceutical ingredients. As far as patient convenience is concerned, chewing gum is discrete and its administration without water promotes higher compliance. Since it can be taken anywhere, a chewing gum formulation is an excellent choice for acute medication. Studies have shown that even non-medicated chewing gums stimulate saliva flow and increases plaque pH, which prevents tooth decay [1, 2]. Today, improved technologies have made it possible to develop and manufacture medical chewing gum with pre-defined properties, such as release of the active substance, taste and texture. A number of active substances in chewing gum formulations are available commercially. Medicated chewing gum is now a well-established dosage form described in the European pharmacopoeia. It offers a number of advantages, including fast onset of action, avoidance of hepatic first pass metabolism for substances absorbed via buccal route and possibly lower dosage requirements and hence a fewer side-effects [3]. Periodontal disease, a common health problem, involves a group of inflammatory conditions due to bacterial etiology affecting the supportive structures of teeth, gingiva, periodontal ligament and alveolar bone. Treatment of periodontitis, an extension of inflammation

into deeper tissues, with conventional root planning and scaling with orally administered antimicrobial agent's results in dose-related undesirable side effects. Larger doses of the drug required for 5 to 7 days of therapy contribute to side effects. Oral hygiene systems such as mouth rinses are ineffective against periodontal pockets, which are receptive to treatment with local, sustained release, tetracycline-loaded hollow fibers and/or chlorhexidine slabs [4]. Mouth infections, such as gingivitis and stomatitis, are mostly caused by aerobic and anaerobic microbes and can be treated locally by antimicrobial and anti-inflammatory drugs, usually administered in buccal gel form or as mouthwashes. However, the disadvantage of these deliveries is that they are easily washed away by saliva, and the effective drug levels in the mouth are limited to a short period of time, necessitating repeated administration [5]. Complementary medicine received a great attention during recent decades which recommends supplementation with various ingredients. Diverse modes of delivery including chewing candy, chewing gum, dentifrices and local drug delivery strips are introduced [6-9]. Tea and in particular, green tea are among most popular beverages, with high daily consumption in Asia and especially in India. Several properties including antioxidant, anticaries, antibacterial, antiviral, ant diabetic, anti-mutagenic and antitumor properties are addressed for green tea [6]. Green tea, *Camellia sinensis* from the family of Theaceae [10] is mostly cultivated in several parts of India. Its remedial effects are associated with the polyphenol contents comprising catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). The two latter are mainly found in green tea rather than the black tea and are among most potential contents to be reviewed for periodontal adjunct therapies in terms of their special anti-collagenase activity [11, 12]. In addition, it is suggested that EGCG inhibits the growth and cellular adherence of periodontal pathogens [13]. To overcome the above mentioned problems in the present study medicated chewing gums were prepared using EGCG (epigallocatechin gallate) enriched extract of *Camellia sinensis* (Green tea) as it has antibacterial and antiplaque properties, to give better patient compliance compared with mouth rinse in lesser dose when compared with the mouth wash.

## 2. MATERIAL AND METHODS

### 2.1. Materials

Lipton green tea (FSSAI Lic. No.10013022001897 & Batch No. 19FC16) was purchased from local market of Bhopal (MP). Acetonitrile, water, acetone, toluene, formic acid were purchased from Central Drug House Pvt Ltd, Mumbai, India. EGCG was obtained as a gift sample from Evrone life sciences, India. Blood agar media (M073-500G), nutrient broth (GRM666-500G), gum base, xylitol, clove oil, talc, ascorbic acid, glycerin was procured from Central Drug House (P) Ltd. New Delhi. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India. All the chemicals used in this study were of analytical grade.

### 2.2. Methods

#### *2.2.1. Extraction by infusion method (Conventional extraction)*

This process was performed with 2 steps method at different temperature.

Extraction condition-

| | |
|---|---|
| pH | 5-6 |
| Water to tea ratio | 25:1 |
| Particle size of leaf | 1mm |

*2.2.1.1. For enrichment of EGCG follow 2 step procedures*

Ten g of green tea was taken in 250ml preheated water at 50°C for 10 min. the extract was filtered and residue was collected. The residue was taken again and treated with 250 ml preheated water at 80°C for 20 min. Both extracted liquid were dried in rotary evaporator at 55°C individually and dry power was collected and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts [14].

#### *2.2.2. Antibacterial activity*

One liter of nutrient broth (GRM666-500G) was prepared by dissolving 13 g of commercially available nutrient broth in 1000ml distilled water, then Agar 1 (Lot- GRM666) (20gm/1000mL) was added and boiled to dissolve the medium completely. The medium was sterilized by autoclaving at at 121°C (15psi) for 15 minutes.

**To prepare** blood agar media 40.0 grams of blood agar base (M073-500G) was suspended in 1000mL purified /distilled water. The mixture was heated to boil to dissolve the medium completely and sterilized by autoclaving at 15 psi pressure (121°C) for 15 minutes followed by cooling to 45-50°C and aseptically added 5% v/v sterile defibrinated blood. Mixed well and poured into sterile petri plates.

*2.2.2.1. Well diffusion method for S. mutans*

Petri plates containing 20ml nutrient agar medium were seeded with 24hrs culture of bacterial strains (*S. mutans*). Wells were cut and 20µL of the second step extract (of different concentrations- 50,100,150,200µg/mL) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well, ofloxacin was used as a positive control [15].

*2.2.2.2. Well diffusion method for P. gingivalis*

Petri plates containing 20mL media were seeded with 24hr culture of bacterial strains (*P. gingivalis*). Wells were cut and 20µL of the second step extract (of different concentrations- 50, 100, 150, 200µg/mL) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Ampicillin was used as a positive control [16].

### *2.2.3. Preparation of medicated chewing gum*

The first step of gum base was carefully weighed and heated up to 70°C in a water bath. Softening agent, anti-oxidant and *camellia sinensis* (green tea) second step extract etc. were weighed exactly, after that other powders such as sweetening agent and filler were levigated, added to the gum base and mixed well. Finally, flavoring agent was added to the prepared mixture at 40°C and left at room temperature to the cooled and cut into desired shape and size, the composition of formulations was given in table 1 [17].

**Table 1: Formulations of medicated chewing gum**

| Ingredients | Formulations | | | | | | |
|---|---|---|---|---|---|---|---|
| | MCG1 | MCG2 | MCG3 | MCG4 | MCG5 | MCG6 | **MCG6 optimize** |
| Gum base | 400 | 500 | 600 | 500 | 500 | 500 | **500** |
| Softening agent | 140 | 140 | 140 | 160 | 180 | 160 | **160** |
| Filler | 100 | 100 | 100 | 100 | 100 | 120 | **120** |
| Sweetening agent | 20 | 20 | 20 | 20 | 20 | 20 | **20** |
| Anti-oxidant | 1 | 1 | 1 | 1 | 1 | 1 | **1** |
| Flavoring agent | 20 | 20 | 20 | 20 | 20 | 20 | **20** |
| Green tea extract | 200 | 200 | 200 | 200 | 200 | 200 | **200** |

*2.2.3.1. Optimization of chewing gum*

Formulation code MCG1, MCG2, MCG3, MCG4, MCG5, MCG6 were prepared by taking gum base, softening agents, filler, antioxidant, sweetening agent and extract. The formulation was assessed using TA.XT (Texture Profile Analysis) where, formulation code MCG6 showed proper parameters by texture profile analysis as compared to standard (Nicogum 2 mg - Cipla) and *in-vitro* release was properly found out. The good chewability is selected on the basis of chewability. The optimized mass was kept constant for further studies. All ingredients were kept constant only changes is made on two ingredients which is gum base and softening agent on the basis of TPA of standard 70% was close to standard so it is further optimized as shown in table 2. For gum base- 495(-1) low, 500(0) medium, 505(+1) high For softening agent-155(-1), 160(0), 165(+1).

### *2.2.4. Evaluation of medicated chewing gum*

*2.2.4.1. Texture profile analysis*

TPA contains many parameters like hardness, adhesives, cohesiveness etc. It contains flat cylinder which is used to compress a sample twice so that it move backward as well as forward to semblance the action of jaw. Bourne proposed later that a single axis compression which is simpler, and now-a-days most experiments are performed using this analysis [18].

The parameters derived from TPA test are as follows.

**Table 2: Optimization of medicated chewing gum**

| Sweetener | Gum base | Softening agent | Antioxidant |
|---|---|---|---|
| 20 | -1 | -1 | 1 |
| 20 | 0 | -1 | 1 |
| 20 | +1 | -1 | 1 |
| 20 | +1 | 0 | 1 |
| 20 | +1 | +1 | 1 |
| 20 | 0 | 0 | 1 |
| 20 | 0 | +1 | 1 |
| 20 | -1 | 0 | 1 |
| 20 | -1 | +1 | 1 |

Cohesiveness = Area $_2$ /Area $_1$ ($A_2$ /$A_1$)
Springines = Time $_2$ /Time $_1$ ($T_2$ /$T_1$)
Gumminess = Hardness x Cohesiveness
Chewiness = Gumminess x Springiness

A) **Hardness-** Hardness is defined as the maximum peak force during the first compression cycle (first bite) and often been substituted by term firmness.
B) **Fracturability** (Brittleness) - Fracturability is defined as the force at the first significant break in the TPA curve.
C) **Adhesiveness –** Adhesiveness is defined as negative force area for the first bite and represents the work required to overcome the attractive forces between the surface of the food and the surface of the other material with which the food comes into contact, i.e. the total force necessary to pull the compression plunger away from the sample.
D) **Elasticity** (Springiness) - It is related to the height that the food recovers during the time that elapses between the end of first bite and the start of the second bite.
E) **Cohesiveness** - It is defined as the ratio of positive force area during the second compression to that during the first compression and may be measured at the rate at which material disintegrate under mechanical action.
F) **Stringiness -** It is the distance the product is extended during decompression before separating from compression probe.
G) **Chewiness -** It is measured in terms of the energy required to masticate a solid food and is calculated as the product of Hardness x Springiness x cohesiveness. It should be calculated in TPA of solid food.
H) **Gumminess-**It is calculated as the product of Hardness x Cohesiveness and it is characteristic of semisolid food, with low degree of hardness and high degree of cohesiveness.

### *2.2.5. In-vitro drug release*

Artificial saliva of pH 6.8 was prepared and 4ml of the same was filled into cavity of *In-vitro* chewebility apparatus, the chewing gum was put into saliva filled in chewebility apparatus release was seen at 60 rpm with time interval of 1,2,5,10,15 min. The sample was analysed by UV spectrometer at 278nm [19].

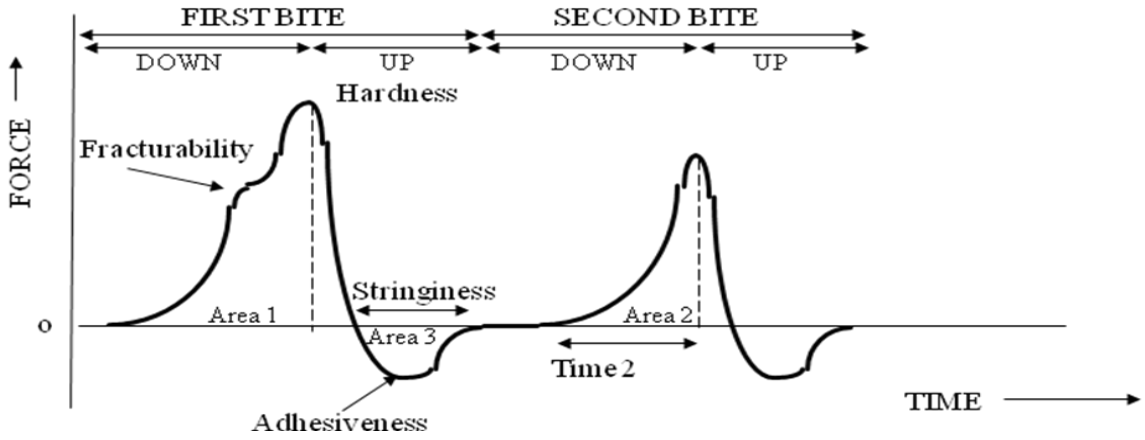


**Fig. 1: Texture profile analysis (TPA) graph showing various TPA parameters**

## 3. RESULTS AND DISCUSSION

In this study overall extractive yield obtained from two step method, the extract obtained with second step was found significantly enriched with greater amount of EGCG per gram of the extract. We found that overall two step procedure was able to enrich the extract with EGCG. As well as 2$^{nd}$ step of two step procedure was able to enrich the extract with EGCG in greater way by such we can get EGCG with more purity. The percentage yield of extracts in first step and in second step was found to be 9.43%, 11.65% respectively. The antibacterial activity was performed by using 24hr culture of *P. gingivalis* and *S. Mutans* by Well diffusion method. There were four concentration used which are 50, 100, 150 and 200µg/ml for extract phytochemicals in antibiogram studies. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Ofloxacin (10µg/ml) and

Ampicillin (10µg/ml) was used as a positive control. It was clear from the experimental data presented in Table 3, 4 & Fig. 2 & 3 that antibacterial activity of extract against *P. gingivalis* and *S. Mutans* in concentration dependent manner as compared with the standard.

**Table 3: Zone of inhibition with extract and ofloxacin against *S. mutans***

| S. No. | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Second step extract concentration µg/ml | | | | Ofloxacin (10µg/ml) |
| | 50ug/ml | 100ug/ml | 150ug/ml | 200ug/ml | |
| 1. | 07.00 | 10.00 | 12.50 | 18.00 | 25.00 |
| 2. | 08.00 | 12.00 | 16.00 | 21.00 | |
| 3. | 10.00 | 13.00 | 15.30 | 22.00 | |
| Mean | 8.33±1.52 | 11.66±1.527 | 14.6±1.85 | 20.33±2.08 | |

**Table 4: Zone of inhibition with extract and Ampicillin against *P. gingivalis***

| S. No. | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Second step extract concentration µg/ml | | | | Standard (Ampicillin 10µg/ml) |
| | 50ug/ml | 100ug/ml | 150ug/ml | 200ug/ml | |
| 1. | 07.40 | 10.00 | 12.30 | 19.00 | 23.00 |
| 2. | 07.00 | 07.60 | 10.00 | 13.00 | |
| 3. | 09.00 | 12.10 | 13.00 | 18.00 | |
| Mean | 7.8±0.86 | 9.9±1.83 | 11.76±1.28 | 16.66±2.62 | |

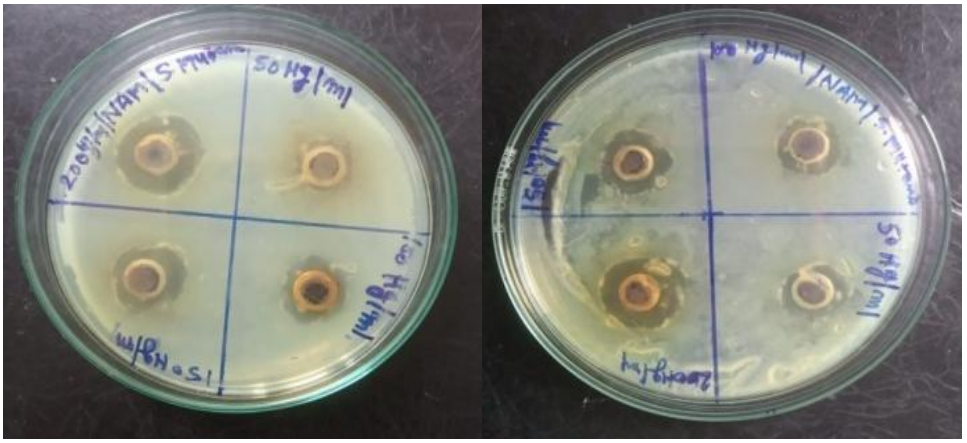

**Plate 1** **Plate 2**

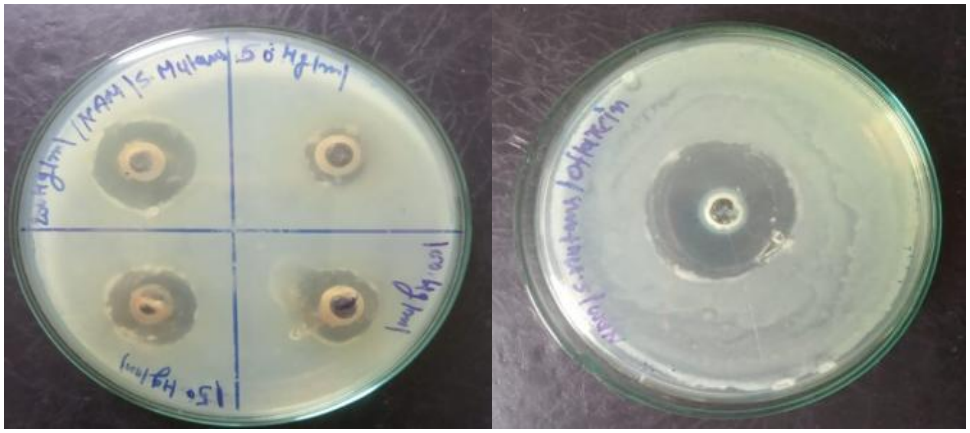

**Plate 3** **Plate 4 Standard (Ofloxacin)**

**Fig. 2: Zone of inhibition with extract and ofloxacin against *S. mutans***

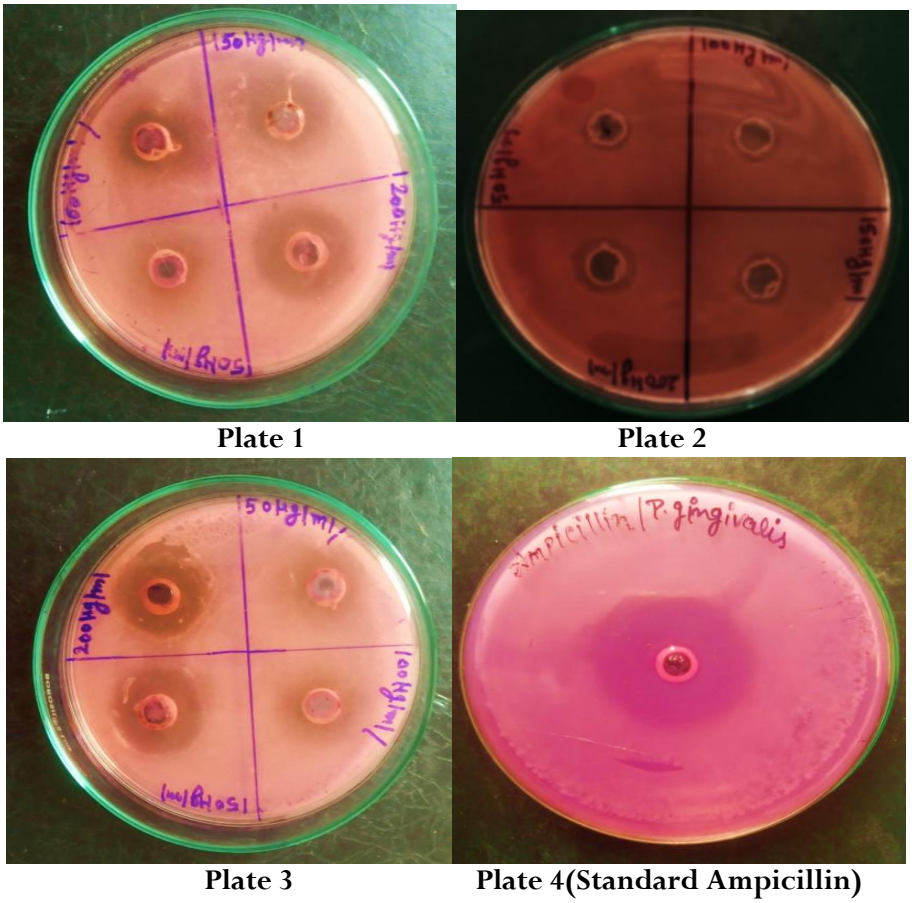


Plate 1 Plate 2

Plate 3 Plate 4(Standard Ampicillin)

**Fig. 3: Zone of inhibition with extract and Ampicillin against *P. gingivalis***

This new formulation "medicated green tea chewing gum" was designed particularly for periodontal disease and dental caries. Chewing gum as a drug delivery system has gained wide acceptance only within smoking cessation & oral health care, clinical trials have proven that there are therapeutic advantages to be gained by using chewing gum as a drug delivery system through exploiting the effects achieved by chewing gum, the convenience of the drug delivery system, & the possibilities of having buccal absorption or local effect of an active substance. Furthermore, one of the trials has indicated that chewing gum as drug delivery systems are possibly safer for active substances that are susceptible to abuse. Chewing formulation may also be less prone to accidental overdose. Medicated Chewing gum formulation was done which was containing *Cameliia sinensis* second step extract (79.78% EGCG). The texture of medicated chewing gum is analysed by texture profile analysis. The analysis of medicated chewing gum is fulfilled all the parameters and it was confirmed by comparing its test with formulation (Nicogum). The results are shown in Fig. 4, 5 & Table 5, 6.

**Table 5: TPA parameters of marketed formulation for the evaluation of nicotine chewing**

| Parameters | NCG1 | NCG2 | NCG3 |
|---|---|---|---|
| **Hardness** | 6104.637 | 7056.251 | 5862.452 |
| **Adhesiveness** | 0.007 | 0.044 | 0.005 |
| **Elasticity** | 0.425 | 0.500 | 0.535 |
| **Cohesiveness** | 1903.699 | 2368.382 | 1802.990 |
| **Chewiness** | 819.540 | 1184.191 | 964.390 |
| **Resilience** | 0.327 | 0.375 | 0.333 |

Chicle gum base is used as natural gum base for medicated chewing gum. The prepared formulation was evaluated by various parameters. The following conclusion can be drawn from the result obtained:

1) Thickness of chewing gum from all formulation found within limit.
2) Stickiness of the formulation decreases with increase in conc. of plasticizer.
3) As the conc. of plasticizer decreases, hardness of medicated chewing gum increases.

The main reason for taking this formulation is that it

contains fewer side effects (almost nil). Catechins are the main ingredient specially EGCG in *Camellia sinensis* (green tea) which is responsible for periodontal disease and dental caries.

The *in-vitro* release of formulated medicated chewing gum was done by *In-Vitro* chewebility machine to compare with Nicotine chewing gum. The *in-vitro* release of medicated chewing gum was found to be 77.26% as compared to std. Nicogum 2 mg (Cipla) Table 7 & Figure 6.

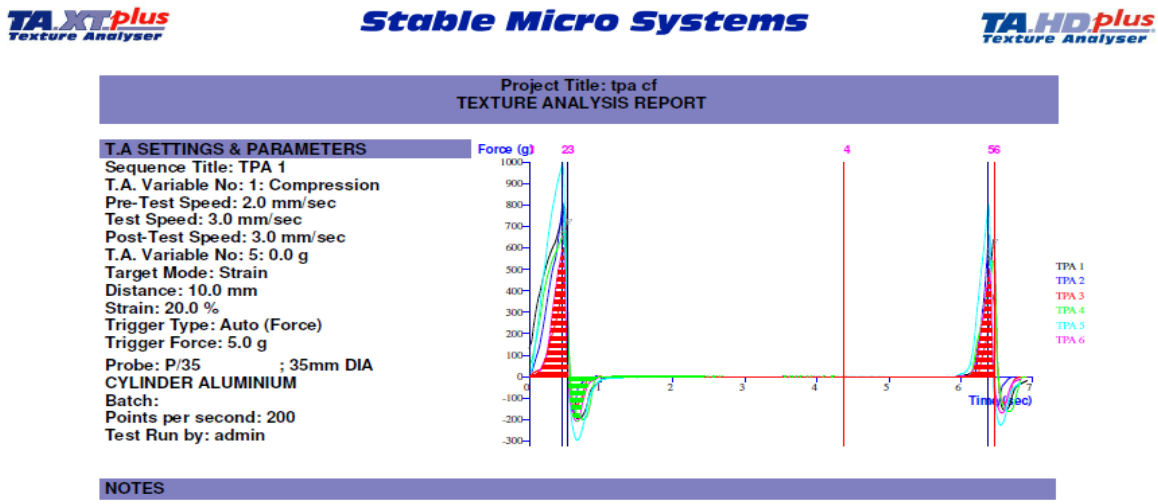


**Fig. 4: TPA report of marketed formulation of nicotine chewing gum**

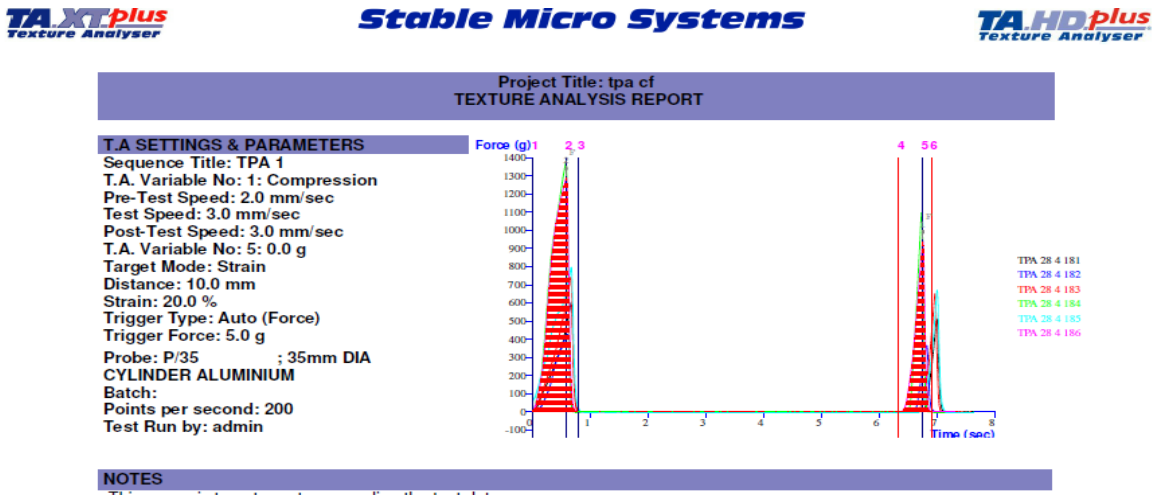


**Fig. 5: TPA report of six formulation of medicated chewing gum**

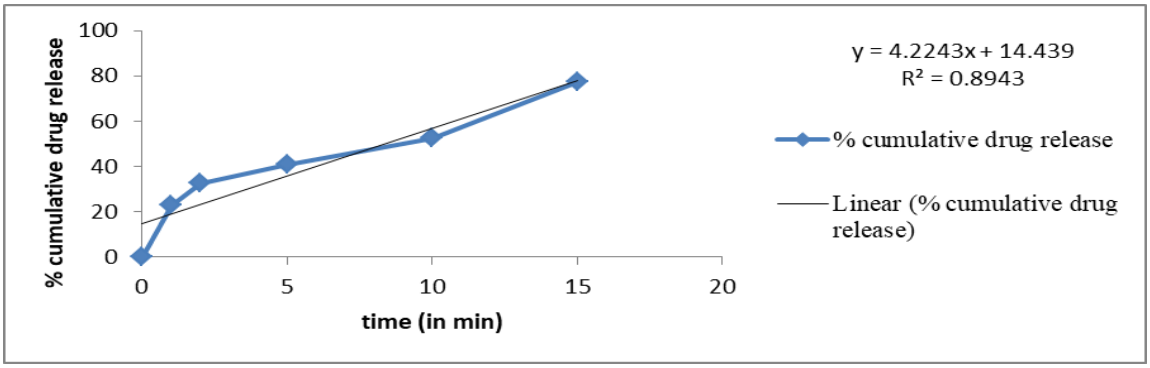


**Fig. 6: indicates the % cumulative drug release by showing calibration curve using UV spectroscopy**

**Table 6: TPA parameters for the evaluation of medicated chewing gum**

| Parameters | MCG1 | MCG2 | MCG3 | MCG4 | MCG5 | MCG6 |
|---|---|---|---|---|---|---|
| **Hardness** | 611.002 | 328.026 | 641.684 | 1277.564 | 694.814 | 1189.653 |
| **Adhesiveness** | - 7.560 | - 11.750 | - 13.219 | - 13.761 | - 27.068 | - 1.017 |
| **Elasticity** | 0.415 | 0.617 | 0.538 | 0.277 | 0.332 | 0.537 |
| **Cohesiveness** | 0.266 | 0.300 | 0.298 | 0.231 | 0.0.308 | 0.467 |
| **Chewniness** | 112.321 | 112.797 | 178.273 | 162.145 | 119.015 | 423.242 |
| **Gumminess** | 218.976 | 161.344 | 194.886 | 356.385 | 202.456 | 505.562 |
| **Resilience** | 0.129 | 0.239 | 0.133 | 0.209 | 0.181 | 0.309 |

**Table 7: Percent cumulative drug release by using *in vitro* chewing gum apparatus**

| Time (in min) | % Cumulative drug release |
|---|---|
| 1 | 21.846±0.16 |
| 2 | 33.622±0.17 |
| 5 | 41.988±0.18 |
| 10 | 53.507±0.19 |
| 15 | 77.264±0.20 |

## 4. CONCLUSION

We have developed the medicated chewing gum by EGCG enriched extracts from green tea. The developed formulation was found similar in all aspects with respect to well establish marketed formulation (Nicogum). This developed formulation may be attractive to the pediatric patients as promising treatment of carries and other oral infection. MCG can increase patient compliance and patient acceptance as well as increase the bioavailability of EGCG enriched extracts from green tea as it showed significant permeation through buccal mucosa. However, clinical pharmacokinetic data are needed to prove it further.

***Conflicts of Interst***

None declared

***Source of Funding***

None declared

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