ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Human Journals
**Research Article**
January 2016 Vol.:5, Issue:2


# Formulation and Characterization of Antimicrobial Oral Gel from Some Herbal Extracts for Treatment of Periodontal Diseases

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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**Reenu Yadav*, Udit N. Soni, Jay Ram Patel, Sunil Singh and Shailendra Modi**

*Oriental College of Pharmacy, Bhopal, India.*

**Submission:** 9 January 2016
**Accepted:** 15 January 2016
**Published:** 25 January 2016



**Keywords:** *Butea monosperma*, *Cordia oblique*, antimicrobial, oral gel

**ABSTRACT**

Purpose: The aim of the present work was to develop an oral gel for brushing with antimicrobial activity which will cure/protect from various periodontal diseases such as periodontitis, gingivitis, and pyorrhea. Methods: Plant materials procured from local suppliers, extracted and standardized. Screening of antimicrobial activity was carried out with the help of disk diffusion method. Gel was formulated by dried extracts of *Butea monosperma*, and *Cordia obliqua.* Gels evaluated on various parameters and standardization of the formulation was performed. Release of drugs was studied at pH 6.8 using a mastication device. Total phenolic and flavonoid contents were estimated by folin-Ciocalteu and aluminum chloride method, and stability studies were performed (40oC and RH 75% ± 5% for 90 days) to assess the effect of temperature and humidity on the concentration of phenolic and flavonoid contents. The results of accelerated stability conditions were compared with that of samples kept under controlled conditions (RT). The control samples were kept at room temperature (25oC, 35% RH for 180 days. Results: Results are encouraging; extracts possess significant antimicrobial activity at very low concentration (15µg/disc, 20µg/disc and 15µg/disc) on oral pathogenic bacteria. Formulation has optimal characteristics as well as has pleasant appearance, fragrance, texture and taste are highly acceptable by the volunteers. The diffusion coefficient values ranged from 0.6655 to 0.9164. Since the R values of korsmayer papas were close to 1, drug release from formulation follows matrix diffusion kinetics. Hence, diffusion was the mechanism of the drug release. Formulation follows Non-Fickian transport mechanism. Most formulations released 50% of their contents within 25-30 minutes. Results obtained from accelerated stability studies are indicative of a slight reduction in flavonoids and phenolic contents with time for long time storage. When measured degradation under ambient conditions, degradation was significantly lower than in accelerated stability study. Conclusion: Plant extracts possess compounds with antimicrobial properties, can be used. Developed formulation will cure/protect from various periodontal diseases. Further development and evaluations oral gel including the isolated compounds on commercial scale and their clinical and toxicological studies are the future challenges.

## INTRODUCTION

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. The association between oral diseases and the oral microbiota is well established. Several agents are commercially available and these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhoea and tooth staining. Development of bacterial resistance to presently available antimicrobial agents and their side effects has necessitated the search for new antimicrobial agent. Hence, the search for alternative products over synthetic continues and natural, plant extracts and phytochemicals isolated from plants used as traditional medicines are considered as good alternatives.[1] It was considered worldwide to explore Indian traditional medicinal plants for development of herbal antimicrobial oral gel ( as a novel drug delivery system). The aim of the present work was to develop an oral gel with antimicrobial activity which will cure/protect from various periodontal diseases such as periodontitis, gingivitis, and pyorrhea. Advantages of oral gels over conventional drug delivery systems includes rapid onset of action, easy consumption, higher patient compliance, fewer side effects like dry mouth and decrease in toxicity.[2] There are several Indian medicinal plants available which are responsible protection from some dental pathogens. A survey was done in the different part of the country to study these plants.

In district Chhatarpur of Madhya Pradesh, many tribal and rural people of villages significantly rely on the local plant resources for their primary healthcare needs. For improving and promoting oral hygiene they use a large number of plants. According to the survey in different parts of Chhatarpur has been documented 40 species belonging to 26 genera and 22 families, being used for dental and oral healthcare. Many plants can be used as chewing sticks or in curing the problems like toothache, bleeding, foetid smell of mouth etc.

## MATERIALS AND METHODS

For the survey, standard method used and advised by Jain (1991) was followed. Information was collected from different tribal people as well as villagers information about the formulations used by the tribals and rural people were collected by the investigators from different study sites of Chhatarpur district in MP. Work was made after careful planning field trips. During the field trips interview were conducted by the author with the informants viz. The tribal groups (Gond,

Kondar, Nat, Barias) and healers (Vaidyas). For compilation of information of the plants used for oral care in Chhatarpur, a literature survey was also carried out.

Plant material was selected on the basis of the survey and authenticated by taxonomist Dr. Manjusa Saxena. Extraction of plant materials was done by methanol followed by preliminary investigations (Physical characteristics and qualitative chemical tests) and standardization of extracts. [3] Screening of antimicrobial activity was carried out with the help of disk diffusion method against some gram-positive (*Streptococcus mutans, S. mitis* and *S. sanguis*), gram negative (*A. actinomycetemcomitans, P. gingivalis* and *B. forsythus*) and fungal strain (*Candida albicans*). [4,5] Minimum inhibitory concentration assay was performed by agar dilution method recommended by the National Committee for Clinical Laboratory Standards. [6] Dried extracts of *Butea monosperma*, and *Cordia obliqua*, sucrose, glycerol, gelling agent, dried extract of *Cuminum cyminum* as flavoring and coloring agents were added in the formulation [7]. Organoleptic characterization was performed at every stage of the development of the formulation. Developed formulation was subjected to further evaluation of various parameters.

**Drug- Excipient Compatibility study by Differential scanning calorimetry**

A differential scanning calorimetry (JADE DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug-excipient compatibility. [8]

**pH:** pH was tested by dissolving 1 ml product into 9 ml of water and shaked vigorously then aqueous solution and pH is observed by pH meter.[9]

**Fragrance test:** It was based on individual observation for its acceptability. Five volunteers were asked for acceptability of fragrance and their opinion was taken, and fragrance was evaluated.

**Various extraction values and ash values:** They were determined i.e. Water soluble extractive, Extraction with Ethanol, Petroleum soluble extractives, Chloroform-soluble extractives. Total Ash value Acid insoluble ash

**Storage stability**: The product was packed and stored for 90 days at each of 5$^{o}$C, room temperature and 40$^{o}$C. The pack was then opened and observed for any change in its physical characteristics occurred or not. Its component changed in colour, odour and consistency were

observed manually. [9] Standardization of the formulation was performed by taking marketed gel as standard formulation. Release of drugs was studied at pH 6.8 using a mastication device. Total phenolic and flavonoid contents were estimated by folin-Ciocalteu [13] and aluminium chloride method. [14] Stability studies were performed (40$^{o}$C and RH 75% ± 5% for 90 days) to assess the effect of temperature and humidity on the concentration of phenolic and flavonoid contents.

**Estimation of total flavonoids and phenolic content**: The test cell was filled with 50ml of simulated salivary fluid (SSF). The gel was placed in the equipment and the instrument was operated for a period of 60 min at a chewing frequency of 56 strokes/ min, to ensure total release of the drug from the formulation in the simulated salivary fluid. From the dissolution medium 5 ml was withdrawn and volume was made up to 25 ml with SSF and from the absorbance of the resulting solution calculates total flavonoids and phenolic content.

***In-vitro* release:** The test cell of the apparatus was filled with 50 ml of SSF and gel was placed in the apparatus. The apparatus was operated at a chewing frequency of 56 strokes / min. 5ml of the SSF from the test cell is withdrawn at regular intervals of 5, 10, 15, 20, 25 and 30 min. Five ml of fresh SSF is replaced back in the test at every withdrawal of the sample. The volume withdrawn was made up to 25ml using SSF and determined the total flavonoids and phenolic content present in chewing gum formulation.[11,12]

**Stability study (Storage stability):** Gel was stored at 40$^{o}$C and RH 75% ± 5% for 90 days. Estimation of flavonoids and phenolics was performed at zero period and then samples were withdrawn after every 18 days. Total 5 samples were withdrawn.

Gel was refluxed with distilled water and ethanol (75 ml) for 30 min. For complete extraction of flavonoids and phenols and filtered through sintered glass funnel by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected in 100 ml volumetric flask and volume was made up with water. The same procedure was performed for each sample and solutions (100 ml) of their total phenolic and flavonoids content were determined. [15, 9, 16]

## RESULTS AND DISCUSSION

Results are encouraging, as all other antibiotics were inactive against these strains, The present study suggests that methanolic extract from seeds and leaves of *Cordia obliqua,* twigs and barks of *Butea monosperma* and seeds of *Cuminum cyminum* possess significant antimicrobial activity at very low concentration (15µg/disc, 20µg/disc and 15µg/disc) on oral pathogenic bacteria. Use of methanolic extracts of these plants as a potential antimicrobial agent in prevention of oral infections and diseases has been suggested. Qualitative chemical tests shown presence of flavonoids, phenolics in the extracts, might be responsible for the activity. Organoleptic evaluation (colour, taste) was done by sensory and visual inspection.

**Table 1. Evaluation of physical characteristics of oral gel**

| Chewing gum Formulation | Physical state | Colour | Odour | Taste |
|---|---|---|---|---|
| **F1** | Semi-Solid | Light yellow | Mood elevating | Pleasant |
| **F2** | Semi-Solid | Light yellow | Mood elevating | Pleasant |
| **F3** | Semi-Solid | Light yellow | Mood elevating | Pleasant |
| **F4** | Semi-Solid | Light yellow | Mood elevating | Pleasant |
| **F5** | Semi-Solid | Light yellow | Mood elevating | Pleasant |
| **F6 ( marketed gel)** | Semi-Solid | White | Mood elevating | Pleasant |

As shown in the table the oral herbal gel has so many features. It exhibits good mouth odour preventive effect and a pleasant and mood elevating fragrance as compared to the reference.

**pH:** pH of the gel was found in the range of 7-7.4.

**Fragrance test:** It was based on individual observation for its acceptability. 5 people were asked for acceptability of fragrance and their opinion was taken.

**Table 2. Evaluation of oral gel on Fragrance test**

| S. no. | Evaluation Parameter | Grades on the basis of evaluation criteria | | | | | Reference F6 N |
|---|---|---|---|---|---|---|---|
| 1 | Fragrance | F1 | F2 | F3 | F4 | F5 | A |
| | | A | A | A | A | A | |

Fragrance was good as good as the fragrance of the reference formulation.

**Table 3. Different ash and standardization values of the formulation**

| S.No. | Physical parameters | % w/w (Sample) | % w/w (standard formulation) |
|---|---|---|---|
| 1 | Total ash value | 2.26 | 2.0-2.5 |
| 2 | Acid insoluble ash | 0.46 | 0.5-1.0 |
| 3 | Water soluble ash | 0.58 | 0.5-1.0 |
| 4 | Chloroform soluble extractives | 1.88 | 1.5-2.0 |
| 5 | Ethanol soluble extractives | 3.60 | 3.5-4.5 |
| 6 | Petroleum soluble extractives | 1.20 | 1.0-1.5 |
| 7 | Water soluble extractives | 1.46 | 1.0-1.5 |

**Table 4. Assay of Formulation (Total Flavonoids and Phenolic compounds)**

| S.No. | Formulation | Total Flavonoids (%) | Total Phenolic (%) |
|---|---|---|---|
| 1. | F1 | 70.65 | 16.56 |
| 2. | F2 | 69.98 | 17.58 |
| 3. | F3 | 72.98 | 18.56 |
| 4. | F4 | 68.54 | 15.58 |
| 5. | F5 | 69.36 | 16.36 |

In comparison to all formulation, Formulation F3 shows maximum flavonoids and phenolic content 72.98 mg/gm and 18.56 mg/gm respectively. On the basis of results of various evaluation parameters F3 selected as best formulation.

**Table 6. *In vitro* drug release study of poly herbal oral gel formulation**

| Time | Cumulative % of Drug Release | | | | | |
|---|---|---|---|---|---|---|
| (min.) | F1 | F2 | F3 | F4 | F5 | F6 standard |
| 5 | 26.35 | 24.65 | 21.26 | 15.26 | 12.23 | 21.26 |
| 10 | 33.63 | 29.36 | 26.36 | 20.36 | 18.56 | 26.36 |
| 15 | 42.26 | 35.26 | 33.26 | 29.69 | 38.23 | 33.26 |
| 20 | 45.56 | 42.56 | 40.25 | 35.65 | 45.56 | 40.25 |
| 25 | 50.26 | 48.89 | 55.56 | 43.48 | 50.26 | 55.56 |
| 30 | 55.45 | 52.15 | 65.36 | 48.87 | 56.25 | 65.36 |

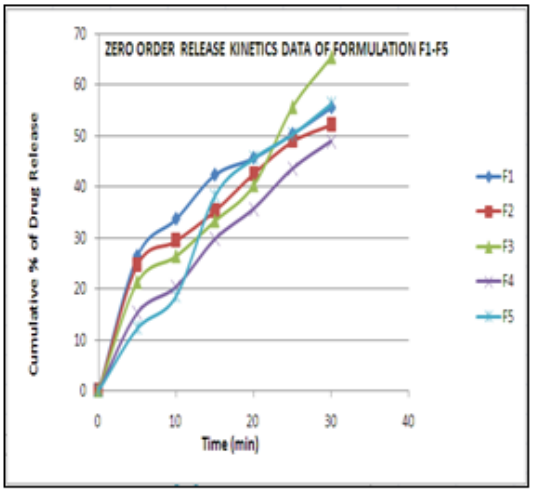


**Fig 1. Zero order release kinetics data of Formulation F1-F5**

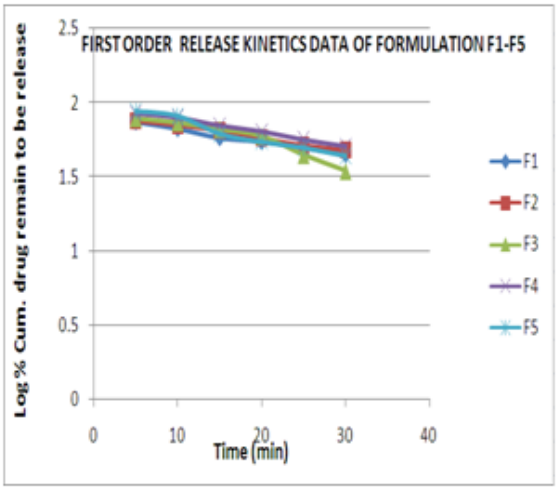


**Fig 2. First order release kinetics data of Formulation F1-F5**

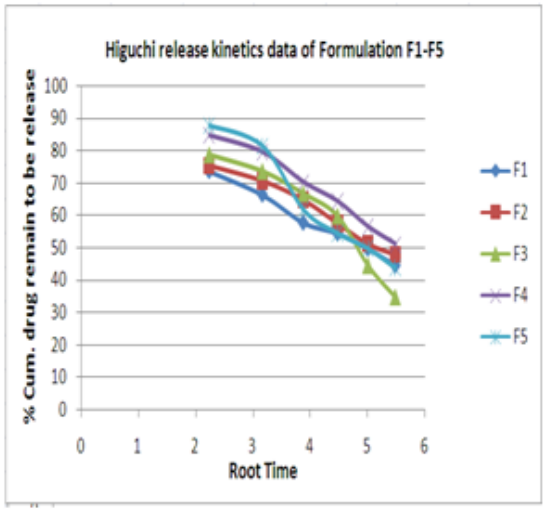


**Fig 3. Higuchi release kinetics data of Formulation F1-F5**

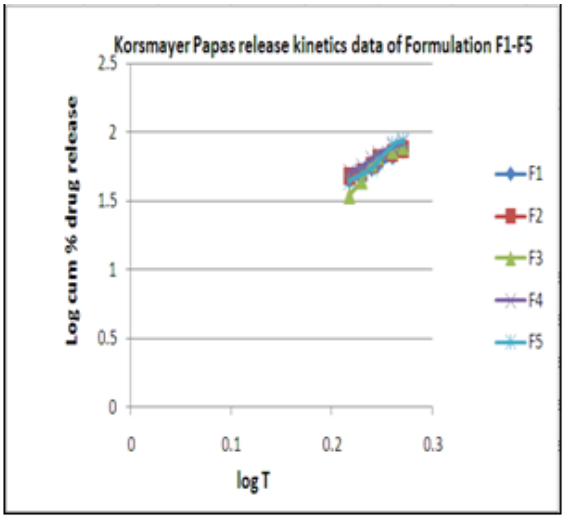


**Fig 4. Korsmayer Papas release kinetics data of Formulation F1-F5**

**Table 7. Kinetic data of polyherbal oral gel formulation in comparison with all formulations**

| FORMULATION | ZERO ORDER | FIRST ORDER | HIGUCHI | KORSMAYER PAPAS |
|---|---|---|---|---|
| **F1** | 0.866 | 0.990 | 0.993 | 0.999 |
| **F2** | 0.900 | 0.990 | 0.977 | 0.969 |
| **F3** | 0.968 | 0.925 | 0.913 | 0.907 |
| **F4** | 0.978 | 0.993 | 0.981 | 0.985 |
| **F5** | 0.963 | 0.968 | 0.964 | 0.978 |
| **F6 Standard** | 0.968 | 0.925 | 0.913 | 0.907 |

**Table 8. Antimicrobial activity of polyherbal oral gel (F3).**

| Name of microorganism | Polyherbal chewing gum (*Cordia obliqua* 2% + *Butea monosperma* 3%extract) |
|---|---|
| | Zone of inhibition (mm) |
| *A.actinomycetemcomitans* | 9.00±0.00 |
| *P. gingivalis* | 9.3±0.62 |
| *Streptococcus mutans* | 9.00±0.34 |
| *S. albony* | 9.8± 0.44 |
| *C.albicans* | 10.00± 0.00 |

Mean, Mean value of diameter of inhibition zone with standard error.

As the diameter of paper disc used was 6mm, 6mm diameter included in the table is indicative of no activity.

**Table 9. Estimation of Total flavonoids content in gel formulation**

| Sample no. | Time interval | Total flavonoids content (% mg/gm) | | |
|---|---|---|---|---|
| | | At $40^0$C | At RT | At $5^0$C |
| 1 | 0 days | 72.98 | 72.98 | 72.98 |
| 2 | 18 days | 71.95 | 72.58 | 72.56 |
| 3 | 36 days | 71.00 | 71.98 | 71.85 |
| 4 | 54 days | 69.56 | 70.54 | 70.54 |
| 5 | 72 days | 68.45 | 69.89 | 68.78 |
| 6 | 90 days | 67.78 | 68.95 | 67.56 |

**Table 10. Estimation of Total Phenolic content in oral gel Formulation**

| Sample no. | Time interval | Total flavonoids content (% mg/gm) | | |
|---|---|---|---|---|
| | | At $40^0$C | At RT | At $5^0$C |
| 1 | 0 days | 18.56 | 18.56 | 18.56 |
| 2 | 18 days | 17.59 | 18.30 | 18.20 |
| 3 | 36 days | 16.90 | 18.12 | 17.56 |
| 4 | 54 days | 16.50 | 17.54 | 17.14 |
| 5 | 72 days | 16.42 | 17.20 | 16.56 |
| 6 | 90 days | 16.00 | 17.10 | 16.15 |

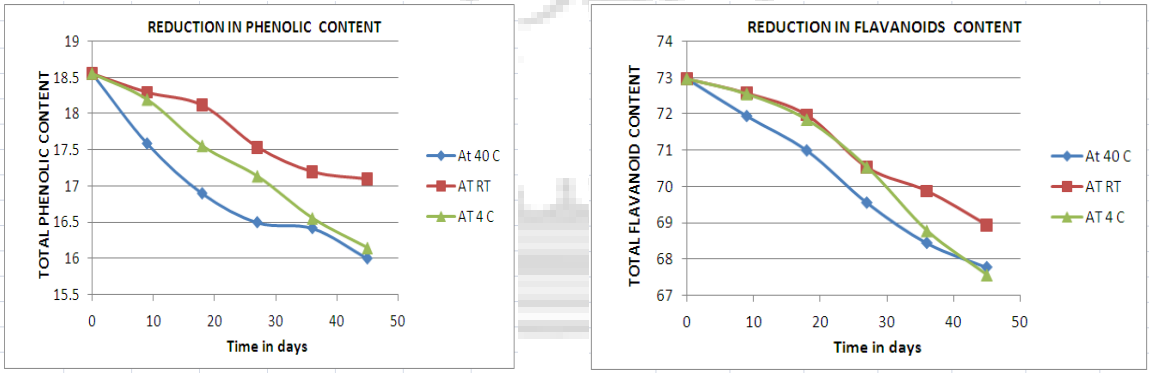


**Fig. 5. Reduction in phenolic and flavonoid contents**

Formulated oral gel has pleasant appearance, fragrance, texture and taste are highly acceptable by the volunteers, as compared to marketed formulation. The mean percentage of drug content in every formulation was found to be in range of 98.2 ± 1.80% to 99.2 ± 0.35%. In all the cases, R values of korsmayer papas model were close to 1. The diffusion coefficient values ranged from 0.6655 to 0.9164. Since R values of korsmayer papas were close to 1, drug release from formulation follows matrix diffusion kinetics. Hence, diffusion was the mechanism of the drug release from the medicated oral gels. Further, observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows Non-Fickian transport mechanism. Most Formulations released 84-89% of their contents within 20 minutes. Results obtained from the accelerated stability studies are an indicative of a slight reduction in flavonoids and phenolic

contents with time for long time storage. Initially on 0 days the concentration of flavonoid and phenolic contents was 72.98 mg/gm and 18.56 mg/gm on 54$^{th}$ day it was observed 69.56 mg/gm and 16.50 mg/gm and on 90$^{th}$ day it was observed 67.78 mg/gm and 16.00 mg/gm from the results it can be concluded that flavonoid and phenolic contents are reducing with time by the effect of the temperature and moisture.

## CONCLUSION

The results of the study support the traditional application of the plants and suggest, plant extracts possess compounds with antimicrobial properties that can be used as potential antimicrobial agents and gels can be a good carrier of herbal extracts. Developed formulation will cure/protect from various periodontal diseases. Further development and evaluations of gels including the isolated compounds on commercial scale and their clinical and toxicological studies are the future challenges

## ACKNOWLEDGMENTS

We thank Mr. Ashish Kesharwani from Cipla Ltd. Mumbai, India. We also like to thank Hi Media Lab Mumbai, India and AIMS, New Delhi for providing us culture media and microbial strains to conduct this research work.

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