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# Formulation and Evaluation of Antimicrobial Mucoadhesive Dental Gel of Aerial Root of *Ficus Benghalensis* L to Enhance the Therapeutic Activity using Clove Oil

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**Abstract:- The main purpose of the research is to develop and evaluate a dental gel containing a herbal medicine for the management of toothache. The ethanolic extract of aerial root of *Ficus benghalensis* was prepared and it was evaluated for organoleptice properties, pH and phytochemical screening. Antimicrobial activity of the extract was done against *streptococcus mutans* using well diffusion method. The gel was prepared using Carbopol, propylene glycol and clove oil as a permeation enhancer. Six gel formulations were prepared at two different grades and various concentration of Carbopol. The formulations were evaluated for different parameters such as appearance, pH, viscosity, spreadability. Theoptimizedbatchwas subjected to further studies via. drug content, stability testing, diffusion study to determine percent cumulative release of drug from gel formulations and antimicrobial study. From the antimicrobial study, it was observed the optimized formulation and marketed formulation produced almost equal zone of inhibition. The evaluation parameters of optimized batch indicate that the prepared dental gel is stable, good drug delivery system and contains antimicrobial agents for the prevention of dental caries and management of tooth ache.**

***Keywords:-*** *Aerial root, Ficus benghalensis, clove oil, dental gel, Carbopol.*

## I. INTRODUCTION

The human teeth function to mechanically break down items of food by cutting and crushing them in preparation for swallowing and digesting. The tooth is one of the body's most unique and complex anatomical and histological components.The deterioration of teeth caused by acids produced by bacteria is known as tooth decay, often known as cavities or caries. The cavities appearin different colours ranging from yellow to black. Pain and eating difficulties are common symptoms [1,2]. Inflammation of the gum tissue around the tooth, tooth loss, infection, and abscess formation are all possible complications.

The cause of cavities is acid from bacteria dissolving the hard tissues of the teeth (enamel, dentin and cementum)[3].Whenever bacteria break down food particles or sugar on the tooth surface, they produce acid. Simple sugars in food are these bacteria's primary energy source and thus a diet high in simple sugar is a risk factor. Diabetes mellitus, Sjogren syndrome, and certain drugs are all risk factors for dry mouth. Antihistamines and antidepressants are two medications that reduce saliva production.Caries is a Latin term that means "rottenness."[4].

Four things are required for caries to form: a tooth surface (enamel or dentin), caries-causing bacteria, fermentable carbohydrates (such as sucrose), and time. This is caused by food sticking to the teeth and the bacteria that make up dental plaque producing acid. Biofilm (dental plaque) that forms on the teeth and matures into cariogenic bacteria causes tooth decay (causingdecay). In the presence of fermentable carbohydrates such as sucrose, fructose, and glucose, certain bacteria in the biofilm produce acid [5-11].

The mutans streptococci, commonly Streptococcus mutans, Streptococcus sobrinus, and Lactobacilli, are the microorganisms most commonly associated with dental cavities.Although there are many different types of oral bacteria in the mouth, only a few are considered to be responsible for dental caries: Among them are Lactobacillus species and Streptococcus mutans. Untreated dental caries can have a negative impact on one's quality of life due to pain, discomfort, tooth loss, and poor oral function. [12]. Pain defines, it is an “unpleasant sensory and emotional experience associated with actual or potential tissue damage”.Dental pain is a common symptom of a variety of dental diseases, including dental caries, and it has a major impact on oral health-related quality of life[13,14].

Patients with tooth pain are generally afraid to take pharmaceutical medicines, instead that preferring to use natural therapies because of their proven efficacy and safety in all age groups.Some studies suggested that the natural herbs can help to prevent the tooth decay and manage the tooth ache[15]. The aerial root of *Ficus benghalensis* has the potent antimicrobial activity which is used for the prevention and treatment of dental plaque, dental caries and periodontitis.

Clove oil is a multipurpose oil that has gained considerable attention in dentistry due to its numerous benefits. Clove oil has a wide range of biological activities, including analgesics, antiseptics, antispasmodics, anti-neuralgics, carminatives, anti-infectious, disinfectant, insecticide, stimulant, stomachic, and others[16].

The goal of this study was to develop a dental gel containing an extract of *Ficus benghalensis* aerial root and clove oil, which acts as a permeation enhancer.

## II. MATERIALS AND METHODS

### A. Materials

The aerial root collected from in and around Tiruchirappalli district, Tamilnadu. The collected aerial roots were authenticated by Botanist, Dept. of Botany, National College, Trichy. Carbopol, Triethanolamine and propylene glycol was a gift sample from Loba chemi, Mumbai. Clove oil was purchased from MSM natural oil and species, Kodaikanal.*Streptococcus mutans- 25175* and *Lactobacillus* was purchased from MTCC, Chandihar, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Himedia, India.

### B. Method of preparation of extract

The aerial root of *Ficus benghalensis* was collected and subjected to shade dry.The material was grinded into a coarse powder after drying in the shade. The preparation of successive solvent extracts was the first step in the process. The dried coarsely powdered sample of *Ficus benghalensis* (90g) was first extracted with petroleum ether (60-80° C) using Soxhlet apparatus and then extracted with ethanol. The extract was subjected to distillation by using simple distillation method. After that, a rotary vacuum evaporator was used to concentrate the extract [17].

### C. Preparation of Gel

Carbopol 940 and 974 were soaked in water for 24 hours before being neutralized with triethanolamine to pH 6.8. Prior to adding Carbopol, a weighed amount of methyl paraben and propyl paraben were added and thoroughly mixed. To obtain a proper gel form, a measured amount of aerial root extract and clove oil were added to the prepared gel, followed by the required quantity of propylene glycol and stirred continuously [18]. To choose the best formulation, all of the prepared gels were subjected through an evaluation test.The various gel compositions were listed in the table 1.

| S.NO | Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| 1 | Extract of aerial root | 2.5 g | 2.5 g | 2.5 g | 2.5 g | 2.5 g | 2.5 g |
| 2 | Clove oil | 0.3 ml | 0.3 ml | 0.3 ml | 0.3 ml | 0.3 ml | 0.3 ml |
| 3 | Carbopol 940 | 0.4 g | 0.6 g | 0.8 g | - | - | - |
| 4 | Carbopol 974 | - | - | - | 0.4 g | 0.6 g | 0.8 g |
| 5 | Propylene glycol | 6 ml | 6 ml | 6 ml | 6 ml | 6 ml | 6 ml |
| 6 | Methyl paraben | 0.18 g | 0.18 g | 0.18 g | 0.18 g | 0.18 g | 0.18 g |
| 7 | Propyl paraben | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g | 0.02 g |
| 8 | Triethanolamine | q. s | q. s | q. s | q. s | q. s | q. s |
| 9 | Distilled water | q. s | q. s | q. s | q. s | q. s | q. s |

Table 1: Formulation of dental gel

## III. EVALUATION

### A. Physical appearance

The physical appearance of the formulation was checked visually

### B. Colour

The formulation's colour was checked against a white background[19].

### C. Consistency

The consistency was checked by applying on skin [19].

### D. Greasiness

The greasiness was assisted by the application on to the skin[19].

### E. Odour

The odour of the gels was evaluated by dissolving them in water and smelling them[19].

### F. pH

2.5 grams of gel were precisely weighed and dispersed in 25 ml of distilled water. A digital pH meterwas used to determine the pH of the dispersion [19].

### G. Homogeneity

The gels were fixed in the container further they were visually inspected for homogeneity and the presence of any aggregate [19].

### H. Spread ability

To determine the spread ability, 0.5g of gel formulation placed between two horizontal smooth surface glass plates (20 cm x 20 cm), the spreading diameter was measured. The initial diameter of the gel on the glass plate was measured in centimetres. For 1 minute, another glass plate (weighing around 200 g) with the same dimensions was placed over the gel until no more expansion was detected. The upper plate was slowly removed, and the diameter of the circle formed after the gel spreaded was measured in centimetres [20].

### I. Viscosity

The viscosities of the prepared gels were determined by using Brookfield viscometer spindle no 7 and spindle speed 60 rpm at 25°C. Viscosities were recorded at room temperature [21].

### J. Drug content

To evaluate the drug content of the gel formulation, the gel (10 mg) was dissolved in about 5-6 ml of phosphate buffer (pH 6.8) and then sonicated for 15 minutes to obtain

complete solubility of the drug. The resulting solution was made up to 10 ml and filtered. The filtrate was then diluted with phosphate buffer 10 times. The drug content was determined by spectro photometry [20].

*K. Extrudability*

The prepared gel was filled into a typical capped collapsible aluminium tube and crimped closed at the end. The tubes' weights were calculated. After being sandwiched between two glass slides, the tubes were clamped. 500g of weight was placed over the slides and then cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel was calculated [19].

*L. In-vitro diffusion study*

1g of the formulation was placed in the donar compartment over an egg membrane that had been washed and immersed in the diffusion medium for 24 hrs. The donar compartment is dipped in the receptor compartment, which contains 400 ml of pH 6.8 phosphate buffer and the beaker holding diffusion medium (receptor compartment) is kept at 37° C with constant stirring at 22rpm using a magnetic stirrer. The study was carried out for 1 hour with 10 minutes interval, 5 ml aliquots are removed from the diffusion medium and replaced with same amount of new, pre warmed diffusion medium. The materials were tested spectrophotometrically at 262 nm using a Shimadzu Double beam UV-visible spectrophotometer [22].

*M. Release Kinetics*

The mechanism of drug release from the dental gel was studied by fitting formulation diffusion data into model-dependent kinetics such as zero order, first order, Higuchi, and Korsemeyer-Peppas equations.

*N. Ex-vivo study*

Ex-vivo buccal permeation tests were performed on goat buccal skin. A 500ml beaker held 400ml of phosphate buffer (pH 6.8) in the receptor compartment. The temperature was kept at 37 ± 0.5°C and the solution was agitated at 900rpm. The gel was placed in Goat buccal mucosal skin and it was tied to the one end of open-ended glass cylinder and then dipped into freshly prepared phosphate buffer on magnetic stirrer. Samples were taken from receptor medium at 0, 10, 20, 30, 40, 50, 60 mins time interval. Periodically 5 ml of sample was withdrawn and same volume of fresh buffer was replaced in the medium.Using phosphate buffer 6.8 pH as a blank, all samples were spectrophotometrically analysed at 262nm.

*O. Antimicrobial activity*

Petri plates containing 20 ml nutrient agar media were injected with bacterial strains that had been cultured for 24 hours (*Streptococcus mutans* and *Lactobacillus*).Wells were cut and concentration of gel formulations were added. The plates were then incubated for 24 hours at 37°C. The diameter of the inhibition zone produced around the wells was used to evaluate antibacterial activity [23,24].

## IV. RESULTS

| Formulations | Appeara-nce | pH | Homogeneity | Spreadability (g-cm/sec) | Extruda-bility (%) |
|---|---|---|---|---|---|
| F1 | Pale yellow | 6.6±0.02 | Good | 18.32± 0.06 | 91.2± 0.21 |
| F2 | Pale yellow | 6.8± 0.05 | Very Good | 16.25± 0.08 | 92.75± 0.31 |
| F3 | Pale yellow | 6.7± 0.04 | Good | 15.78± 0.04 | 89.01± 0.52 |
| F4 | Pale yellow | 6.7± 0.04 | Good | 18.68± 0.05 | 90.44± 0.45 |
| F5 | Pale yellow | 6.8± 0.05 | Very good | 17.88± 0.09 | 93.13± 0.22 |
| F6 | Pale yellow | 6.7± 0.03 | Good | 14.38± 0.05 | 88.5± 0.31 |

Table 2: Properties of dental gel

| **Formulations** | **Viscosity (cps)** | **Drug content (%)** | **% Drug release** |
|---|---|---|---|
| F1 | 8195± 45.12 | 91.3± 0.15 | 87.89± 0.55 |
| F2 | 9450± 67.05 | 95.3± 0.12 | 93.10± 0.52 |
| F3 | 9890± 68.09 | 93.4± 0.35 | 90.45± 0.25 |
| F4 | 9286± 49.68 | 92.2± 0.13 | 92.05± 0.63 |
| F5 | 9550± 55.12 | 95.5± 0.25 | 96.10± 0.07 |
| F6 | 9800± 72.15 | 95.0± 0.41 | 94.89± 0.10 |

Table 3: Characterization of dental gel

*A. Diffusion study plot for formulations*

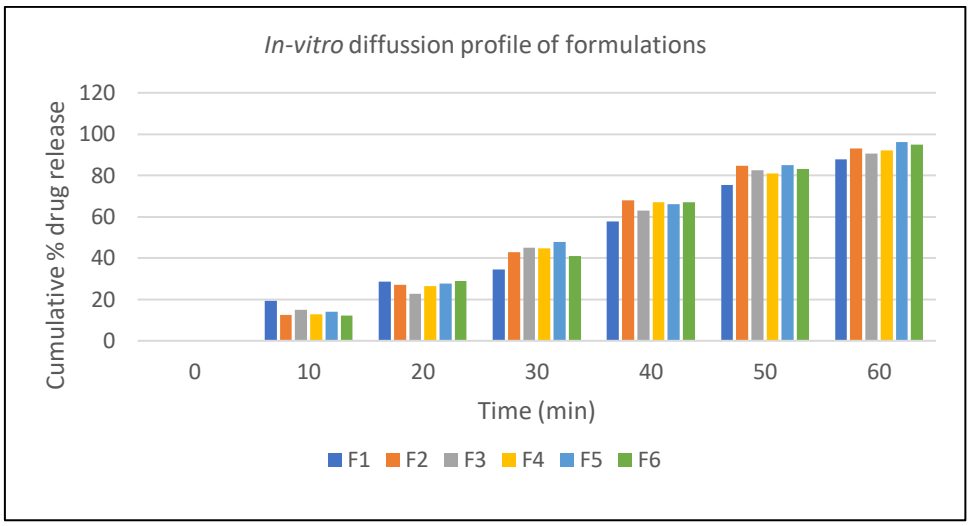
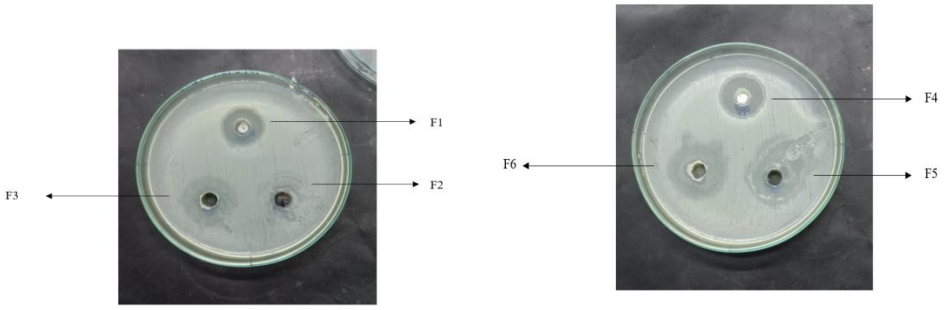



Fig. 1: *In-vitro* diffusion profile of formulations

*B. Antimicrobial activity*

- **Antimicrobial activity gel formulations on *Streptococcus mutans***




- **Antimicrobial activity of gel formulations on *Lactobacillus acidophilus***

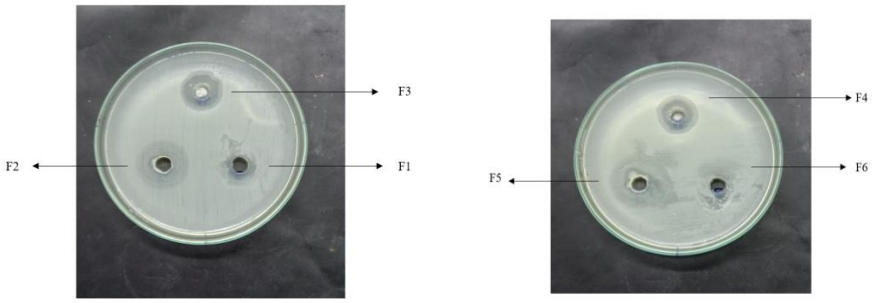



Fig. 2: Antimicrobial activity of formulations on organisms

- **Zone of inhibition of organisms**

| Formulation | Zone of inhibition (*S.mutans*) | Zone of inhibition (*L.acidophilus*) |
|---|---|---|
| F1 | 17.91± 0.05 | 15.23± 0.62 |
| F2 | 21.6± 0.3 | 18.61± 0.3 |
| F3 | 19.82± 0.4 | 17.62± 0.2 |
| F4 | 18.52± 0.01 | 17.55± 0.1 |
| F5 | 22.51± 0.1 | 20.42± 0.05 |
| F6 | 20.02± 0.5 | 18.35± 0.1 |

Table 4: Zone of inhibition of organisms

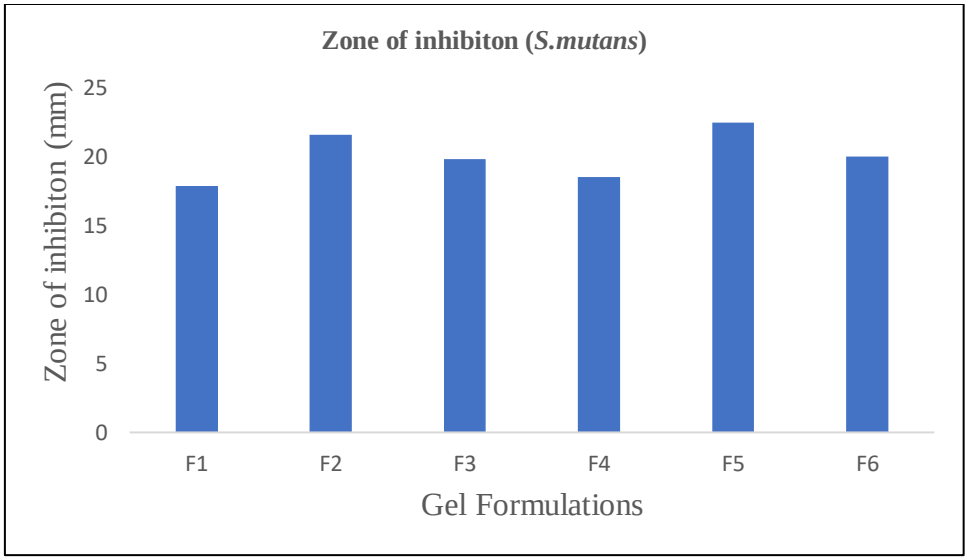



Fig. 3: Zone of inhibition (*Streptococcus mutans*)

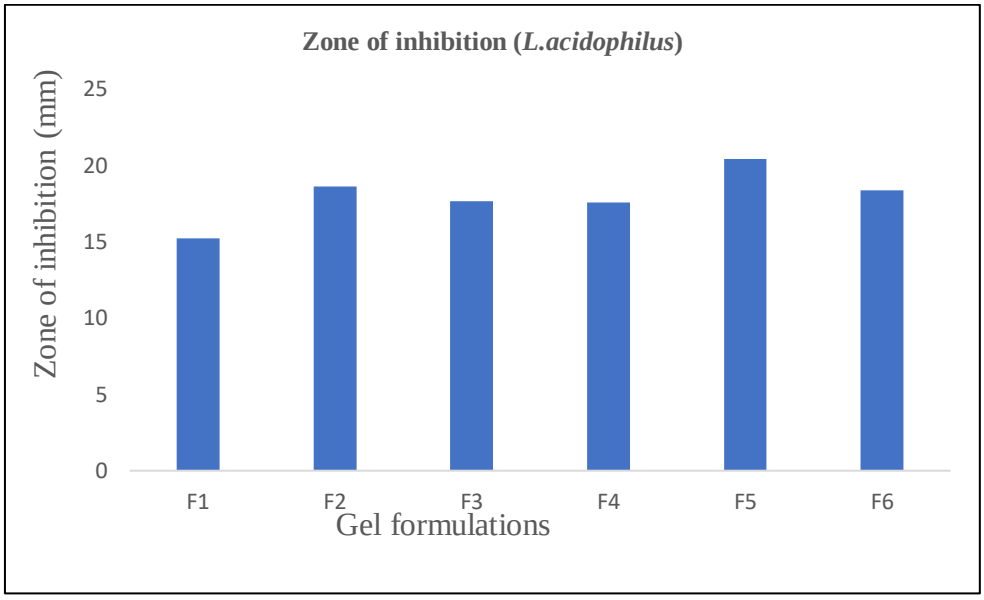
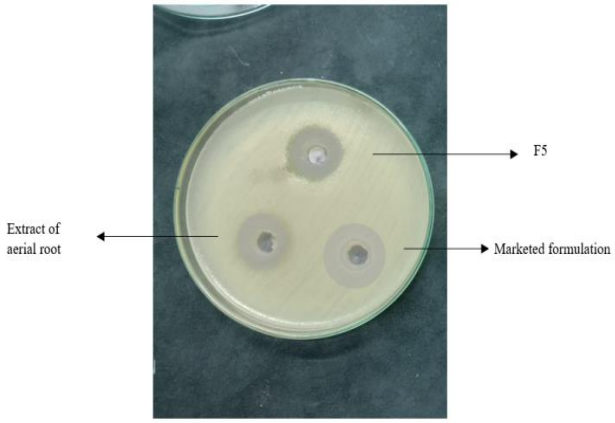



Fig. 4: Zone of inhibition (*Lactobacillus acidophilus*)

- **Comparison of Antimicrobial activity of the formulation F5 with marketed formulation**
- **Antimicrobial activity on *Streptococcus mutans***



- **Antimicrobial activity on *Lactobacillus acidophilus***

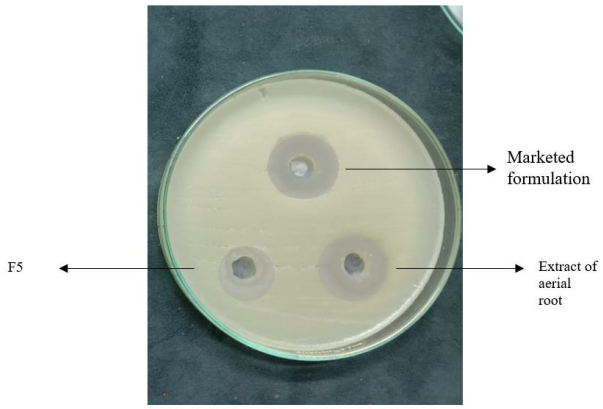



Fig. 5: Comparison of antimicrobial activity of F5 with marketed formulation

| Microorganisms | F5 | Extract of aerial root | Marketed formulation |
|---|---|---|---|
| *Streptococcus mutans* | 22.51± 0.1 | 23.32± 0.4 | 24.6± 1.1 |
| *Lactobacillus acidophilus* | 20.42± 0.05 | 21.01± 0.06 | 21.3± 0.2 |

Table 5: Comparison of Antimicrobial activity of F5 with marketed formulation

## V. DISCUSSION

The formulations were developed by using extract of aerial root of *Ficus benghalensis*using two grades of Carbopol at varied concentrations. The formulation composition was shown in the Table 1. All the six batches of formulations were subjected to physicochemical evaluation. The colour of all the formulations were pale yellow in colour.

As shown in Table 2, the pH of all formulations ranged from 6.6 to 6.8. This was considered to be closer to the pH of the buccal cavity (6–7), suggesting that the gel formulations will be irritation-free.

The homogeneity of all formulations was shown in the Table 2. All the formulations were homogeneous and free of aggregates.

The Spreadability of prepared gels were found to be in the range of 16.25 – 18.68 $g^{-cm}$/sec, confirming that the gel formulations were spread smoothly and uniformly. The tube extrudability of all formulations was good.

The drug content of gel formulations was ranged from 91.3 to 95.5% Table 3. It indicates that there was no degradation of drug during the preparation process.

The *in-vitro* drug release data for the formulation were studied and shown in Table 3. The examination of the regression coefficient value $r^2$ forF5 indicated that the drug release followed diffusion mechanism. From release kinetic results, F5 was selected as the optimized formulationandKorsmeyer -Peppas model release exponent 'n' valuewas 1.106, thus it follows diffusion mechanism. Among the six formulations F5 showed maximum drug release (96.10%).

The gel formulation of extract of aerial root of *Ficus benghalensis* F5 shows good physicochemical properties as well as good drug content compared to other formulations.

All the six formulations were subjected to antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus*. From the report, it has shown that formulation F5 has highest antimicrobial potential which is further compared with marketed formulation (Table No 4).

From the Table No 5, it was observed that optimized formulation and marketed formulation produced almost equal zone of inhibition. Hence, optimized formulation F5 has produced good antimicrobial effect.

## VI. CONCLUSION

The aerial root of *Ficus benghalensis* was found to have antimicrobial activity against S*treptococcus mutans* and *Lactobacillus acidophilus.* The gel formulations developed from aerial root shows significant results so that it can be further used commercially to develop dental gels after conducting theclinical trials on human beings. However, more research is required to identify whether they can effectively replace synthetic antibiotics or be used in combination.

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