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Formulation and Development of Dental Gel Containing Eugenol Oil for the Treatment of Human Gum Diseases

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ABSTRACT

The study was aimed to develop and evaluate dental gel containing Eugenol Oil as the chief constituent for the treatment of Gums. It has a wide spectrum of antibacterial activity against several periodontal Pathogens: hence it is selected for the treatment of Gums. Eugenol Oil gel is formulated by using carbopol 940 as a gelling agent, Eugenol Oil as a medicinal agent, polyethylene glycol co-solvent, methylparaben, and propylparaben as a preservative and required quantity of distilled water as vehicle. Results: The Eugenol Oil was evaluated for physical parameters like acid value, ester value, specific gravity, and refractive index and it showed satisfactory results. The ready gel was evaluated for numerous properties like antimicrobial activity, pH, spreadability, extrudability, drug content, etc. *In-vitro* experiments demonstrated that the formulation F3 is a suitable dosage form for the treatment of Gums. Eugenol Oil showed the zone of inhibition of about 22.05 ± 0.04 mm. Based on the result obtained in this present study, we conclude that the gel formulations of Eugenol Oil F3 showed good physicochemical properties as well as good drug content compared to other formulations.

INTRODUCTION

Periodontal disease is recognized as a major public health problem throughout the world and occurs in all groups, ethnicities, races, genders and socioeconomic levels. It is characterized by inflammation and degeneration of the gums, supporting bone, periodontal ligament and cementum and accumulation of bacterial pathogens, mainly within the periodontal pockets ^[1].

The periodontal disease commonly refers to inflammatory diseases that are plaque-induced i.e. gingivitis and Gums. Gingivitis, the moderate stage of disease caused by an accumulation of supragingival plaque and characterized by swelling, light bleeding, and redness of the marginal gingiva. Gingivitis is associated with a change in the microflora, shifting from a Gram-positive anaerobic flora to a more Gram-negative one. Gums, a more severe stage of periodontal disease, results in the reabsorption of the alveolar bone and detachment of the periodontal ligament supporting tooth ^[2].

Gums is an inflammatory response to the overgrowth of anaerobic organisms such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Prevotella melaninogenica*, and *Actinobacillus actinomycetes* contain. The conventional method of treatment the periodontal disease like oral, topical and systemic dosage forms have major disadvantages like superinfection, low or non-compliance, low gingival crevicular fluid levels of antibiotics, systemic side effects, short duration and high relative cost ^[3].

Periodontal treatment aims to cure inflamed tissue, cut back the number of morbidic bacteria and eliminate the pathologic pockets. Recent advances in the field of dentistry have promoted the use of herbal and natural products for the treatment of various oral diseases. There are varied reports of the development of ancient plants and natural product for the treatment of oral diseases. Eugenol Oil is one such product exhibiting multiple benefits and has gained considerable importance in clinical research ^[4].

Since Eugenol Oil shows low intrinsic toxicity along with a wide spectrum of biological actions like analgesic, antiseptic, antispasmodic, anti-neuralgic, carminative, anti-infectious, disinfectant, insecticide, stimulant, stomachic and other useful properties, it is very useful in dentistry also ^[5].

The present study was aimed to formulate dental gel containing Eugenol Oil for the treatment of periodontal diseases and then evaluated for their physicochemical properties including drug content, spreadability, extrude ability, in-vitro antibacterial activity.

MATERIALS AND METHODS

MATERIALS

Eugenol Oil is purchased from the local market in Nellore.

Methylparaben and propylparaben were procured from S.D. Fine chemicals Pvt. Ltd, Mumbai, India.

METHODS

Method of Preparation of Gel

Carbopol 940 gels were prepared by soaking carbopol 940 in water and by neutralizing with triethanolamine to pH 6.4. A weighed amount of methyl and propylparaben were added to the water before the addition of carbopol 934 [6]. In another beaker, the required quantity of propylene glycol was taken in another test tube to which accurately measured the amount of Eugenol Oil corresponding to its MIC was incorporated and finally, this mixture was added to the beaker containing carbopol with stirring [7]. The sweetening agent was also added to the polymer dispersion and stirred continuously until it forms a homogenous product [8]. The volume was created up with water and stirring was done smartly. All the ready gels were then subjected to analysis tests to pick the most effective formulation. The composition of different gel formulations is listed in Table 1.

Physicochemical characteristics of Eugenol Oil:

Eugenol Oil was analyzed for physicochemical characteristics like acid value, ester value, solubility, density, refractive index, and results were tabulated in Table 2.

Evaluation of gel formulation

Physical appearance:

- **Colour:** The color of the formulation was checked out against a light background.

- **Consistency:** The consistency was checked by application on the skin.
- **Grittiness:** The grittiness was assisted by the application on to the skin.
- **Odor:** The odor of the gels was checked by mixing the gel in distilled water and taking the smell.
- **Determination of pH:** The pH of the gel was check using digital pH meter by dipping the glass electrode completely into the gel system ^[9].
- **Determination of viscosity:** Viscosities of the formulated gels was check using Brooke field viscometer, spindle no. 7 and spindle speed 50rpm at 20°C was used for gels, the corresponding dial reading on the viscometer was noted ^[10] Table 5.
- **Determination of spreadability:**

Spreadability was formed by change picked block and glass slide equipment. The equipment consisted of a picked block with a fixed glass slide and a pulley ^[11]. A pan was attached to another glass slide (move) with the help of a string ^[12-15]. For the determination of spreadability measured amount of gel was placed in the fixed glass slide, the movable glass slide with a pan attached to it, was placed on the fixed glass slide such that the gel was sandwiched between the two slides for 5 minutes. Now about 50 grams of weight was added to the pan ^[16-18]. The time duration for the slides to separate was noted.

Spreadability was determined using the following formula.

$$S = M.L/T$$

- Where, S = Spreadability.
- M = Weight in the pan (tied to the upper slide).
- L = Length moved by the glass slide.
- T = Time (in sec.) taken to separate the upper slide from the ground slide.

Where S is the spreadability in grams.cm/sec, M is the mass in grams: T is the time in seconds.

- **Determination of extrudability:**

It was determined by employing a tube crammed with the gel, having a tip of five mm gap and by activity, the quantity of gel that extruded through the tip once pressure was applied on the tube was noted down.

- **Determination of homogeneity:**

All the developed gels were tested for homogeneity by visual inspection after the gels have been set in the container ^[19-20]. They were tested for his or her look and appearance and presence of any aggregates.

- **Determination of drug content:**

The drug content of the gel formulations makes up my mind by dissolving Associate in Nursing an accurately weighed amount one g of gel in 100 metric capacity unit of solvent (a mixture fermentation alcohol and phosphate buffer pH 6.8 for the formulation of Eugenol Oil). The solutions were kept for shaking for 4 hr and then kept for 6 hr for the complete dissolution of the formulations ^[21]. Then the solutions were filtered through 0.45 mm membrane filters and proper dilutions were made and solutions were subjected to the spectrophotometric analysis ^[22]. The drug content was calculated from the simple regression equation obtained from the standardization information.

- **Determination of antimicrobial activity:**

Agar cup plate method was used for screening of antimicrobial activity of Eugenol Oil gel. All formulations of Eugenol Oil gel of about 2% were placed aseptically in cups of agar plate which was previously inoculated with the culture ^[23]. The plates were left at ambient temperature for 30 mins before to incubation at 37°C for 24 hrs. The broad-spectrum antibiotic i.e., Doxycycline was used as a positive control for obtaining comparative results. Plates were observed after 24-48 hrs incubation for the appearance of the zone of inhibition [. Antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (millimeters) of microbial growth.

Stability study:

The stability study was a determination as per ICH guidelines. The developed gel was stuffed in collapsible tubes and hold on at completely different temperatures and humidness conditions, viz. $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ /hour five-hitter RH, $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / $65\% \pm 5\%$ RH, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / $75\% \pm 5\%$ RH for three months and studied for appearance, pH and spreadability.

***In-vitro* antibacterial activity Method of Anti-bacterial susceptibility testing:**

Antimicrobial status testing ways were adopted by disc diffusion technique. The pathogenic organism was grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth round the disks was Associate in Nursing indirect live of the flexibility of that compound to inhibit that organism known as the zone of inhibition. [18- 21]

Test microorganisms:

The four microorganism strains were utilized in this study that obtained from Department of biology, Institute of Medical Sciences, Banaras Hindu University Varanasi. The bacterial strains used were Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeruginosa. The effects of the formulation on the bacterial strains were assayed by disc diffusion method.

Preparation of Mueller-Hinton Agar Medium:

22.8 gm of MH agar media (purchased from HI media laboratories Pvt. Ltd.) was suspended into 600 metric capacity units distill water and mixed properly. After that, it was heated to dissolve the whole media completely. Sterilized by autoclaving at 15 lbs pressure and 120°C temperature for quarter hours mixed well before use.

Preparation of MH plates:

Approximately 25 metric capacity unit of liquid MH media was poured (to a depth of 4 mm) into Petri plates and allowed to solidify at room temperature, stored at 4 to 80°C temperature hydrogen ion concentration MH agar ought to should fall between 7.2 and 7.4 at room temperature after solidification.

Procedure for disc diffusion test

Inoculum Preparation:

At least 3 to 5 well-isolated colonies of the same morphological kind were elected from Associate in nursing agar plate culture. The top of every colony and also the growth was transferred into a tube containing four to five metric capacity of the unit of an accepted broth medium, such as soy broth. The broth culture was incubated at 35°C until it achieves or exceeds the cloudiness of the 0.5 McFarland standards (usually 2 to 6 hours). The turbidity of the actively growing broth culture was adjusted with sterile saline to obtained turbidity optically comparable to that of the 0.5 McFarland standards.

Inoculation of test plates:

Optimally, the quarter-hour when adjusting the cloudiness of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was revolved many times and ironed firmly on The within a wall of the tube above higher than the fluid level to get rid of excess substance from the swab. The dried surface of a Mueller-Hinton agar plate was inoculation by streaking the swab over the entire sterile agar upper surface. This procedure was perennial by streaking 2 additional times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As the last step, the rim of the agar was swabbed. The lid also is left partially opened for three to five minutes, however less than quarter-hour, to permit for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of discs to inoculated Agar plates:

The disc was immersed onto customary antibiotics (5µg/disc) and placed on the surface of the inoculated agar plates. On the other hand, discs were distributed onto the surface of inoculated agar plates and take a look at formulation batches (F2 & F4, 8µl/disc) was applied to the disc with the help of micropipette. Each disc should be ironed down to guarantee complete contact with the agar surface. The discs were placed individually with the help of forceps they must be distributed evenly so that there were no closer than 24 mm from the center to center. Because no of drug diffuses nearly in a flash, a disc should not be relocated once it has come into contact with the agar surface. The plates were inverted and placed in an apparatus set to 35°C within the quarter-hour when after the discs applied^[22].

Reading plates and interpreting results:

After fourteen to sixteen hours of incubation, each plate was examined. If the plate was satisfactorily patterned, and the inoculum was correct, the resulting zones of inhibition should be uniformly circular and a confluent lawn of growth. The diameters of the zones of completely inhibited (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter with the help of a ruler, which was held on the back of the inverted Petri plates. The Petri plate was held a few inches above a black, non-reflecting background and illuminated with reflected light. The zone margin should be taken because the space showing no apparent, visible growth that can be detected with the unaided eye. Faded growth of small colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, should be ignored. After measured the diameter of zone of inhibition the information was noted and deciphering the result. [23].

RESULTS AND DISCUSSION

RESULTS

Table No. 1: Anti-Bacterial Activity of Eugenol Oil gel formulations Composition of a gel formulation

Ingredients	F1	F2	F3	F4	F5
Eugenol Oil (ml)	0.1	0.1	0.1	0.1	0.1
Carbopol 940 (g)	0.3	0.4	0.5	0.6	0.7
Polyethylene glycol 400 (ml)	15	15	15	15	15
Glycerine (ml)	5	5	5	5	5
Methylparaben (g)	0.18	0.18	0.18	0.18	0.18
Propylparaben (g)	0.02	0.02	0.02	0.02	0.02
Aspartame (g)	0.4	0.4	0.4	0.4	0.4
Distilled Water (ml)	qs	qs	qs	qs	qs

Table No. 2: Physicochemical Characteristics of Eugenol oil & Doxycycline

Sr.No.	Parameter	Eugenol oil (Ref)	Eugenol Oil (Std)
1	Colour	Pale Yellow	Pale Yellow
2	Odor	Aromatic	Aromatic
3	Acid Value	3.66	3.84
4	Ester Value	37.21	38.22
5	Solubility In Ethanol	Freely Soluble	100% Soluble
6	Density	1.02g/ml	1.06g/ml
7	Refractive Index	1.492	1.532

Table No. 3: Antibacterial Activity of Formulation of Eugenol oil

Bacteria	Zone of inhibition in mm(F3)	Eugenol Oil	Doxycycline (standard)
<i>S. salivarius</i>	22.05±0.04 n=3	23±0.2 n=3	25±1.1 n=3
<i>S. sanguis</i>	21.56±0.02 n=3	22±0.04 n=3	23±2.2 n=3
<i>L. acidophilus</i>	20.32±0.4 n=3	20±0.06 n=3	21±1.4 n=3
n=3, Mean + SD			



Figure No. 1: Formulation of Eugenol Oil Gel.

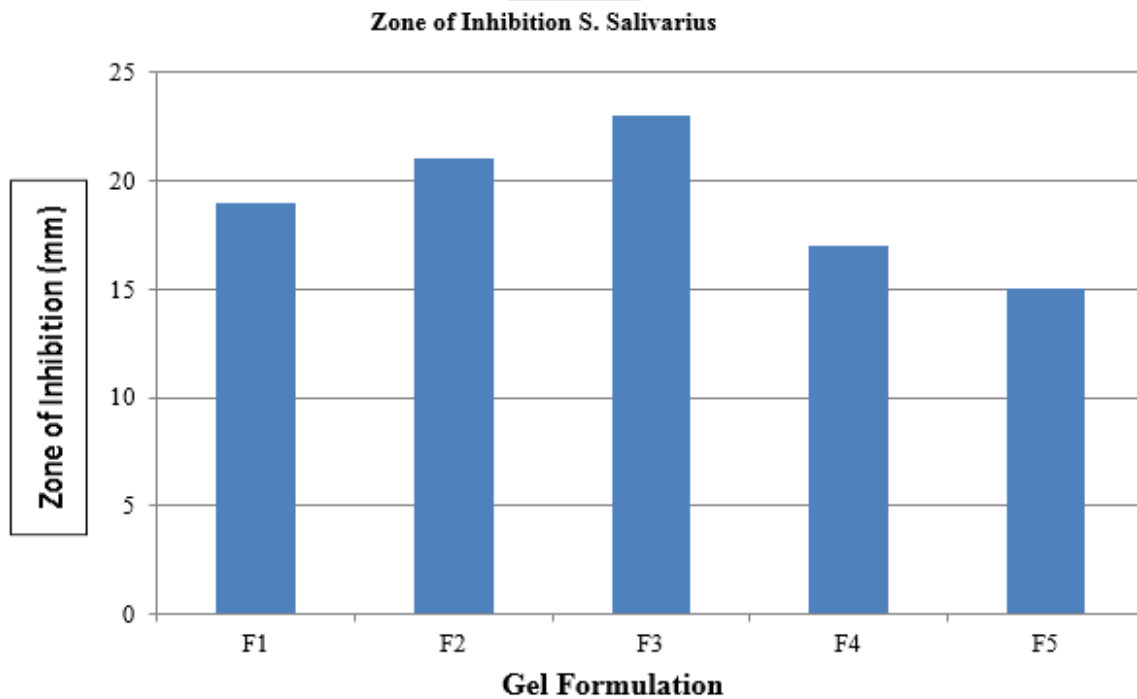


Figure No. 2: Zone of Inhibition *Streptococcus salivarius*

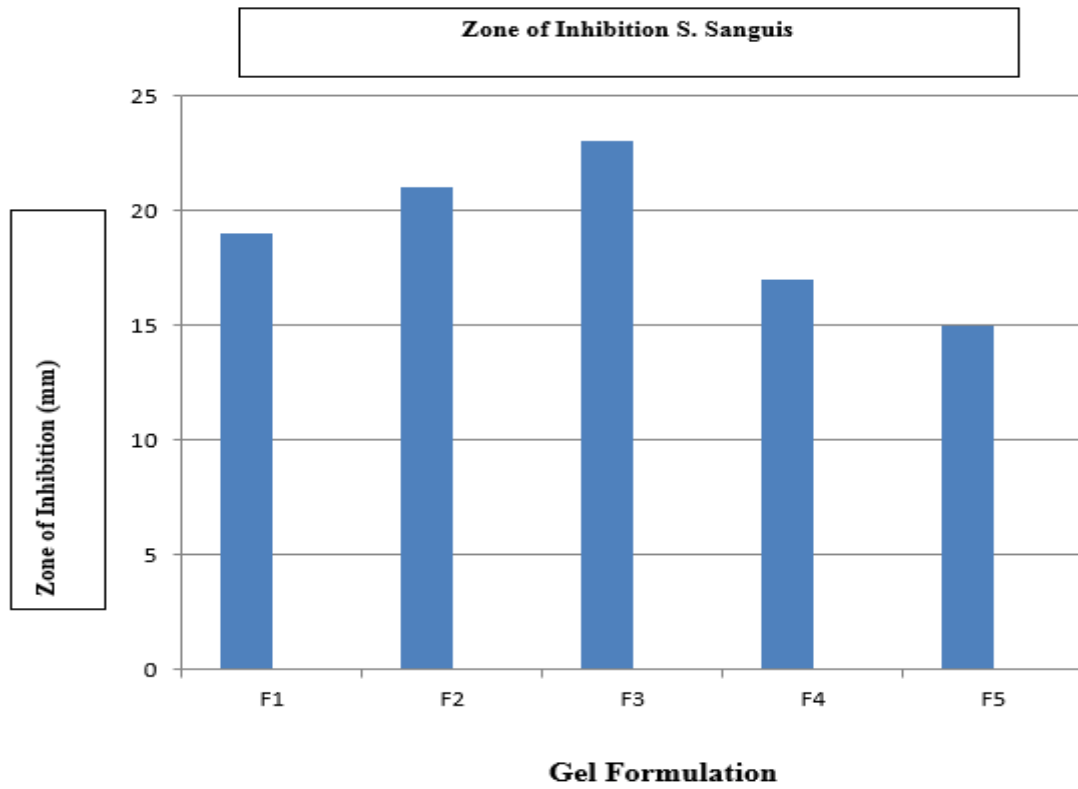


Figure No. 3: Zone of Inhibition Streptococcus sanguis

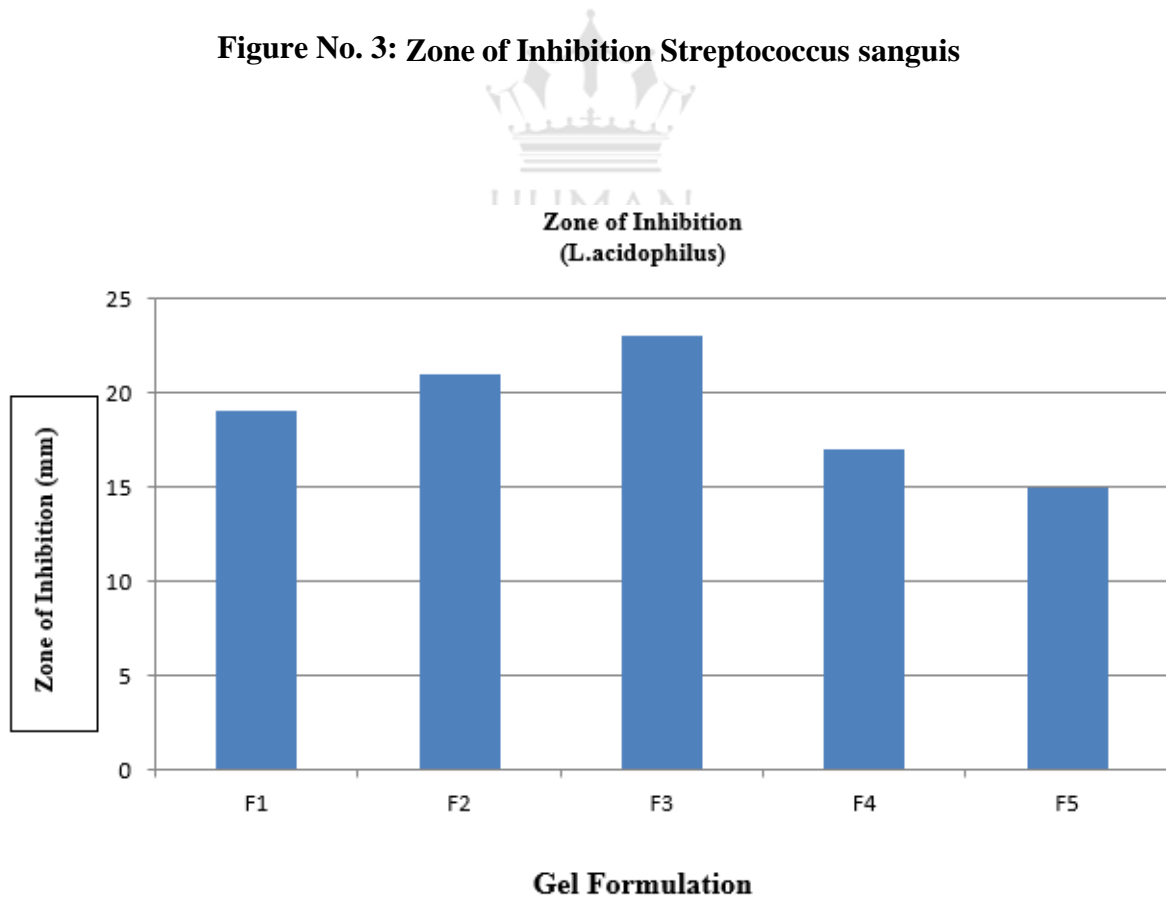


Figure No. 4: Zone of Inhibition of Lactobacillus acidophilus

Table No. 4: Anti-Bacterial Activity of Eugenol oil Gel Formulation

Formulation	Zone of inhibition (S. salivarius)	Zone of inhibition (S. sanguis)	Zone of inhibition (L. acidophilus)
F1	19.53±0.3	18.24±0.2	17.52±0.5
F2	21.5±0.2	20.3±0.1	19.29±0.2
F3	22.05±0.04	21.56±0.02	20.32±0.4
F4	18.6±0.1	18.1±0.03	17.59±0.1
F5	17.06±0.02	16.13±0.4	15.43±0.6
n=3, Mean±S.D			

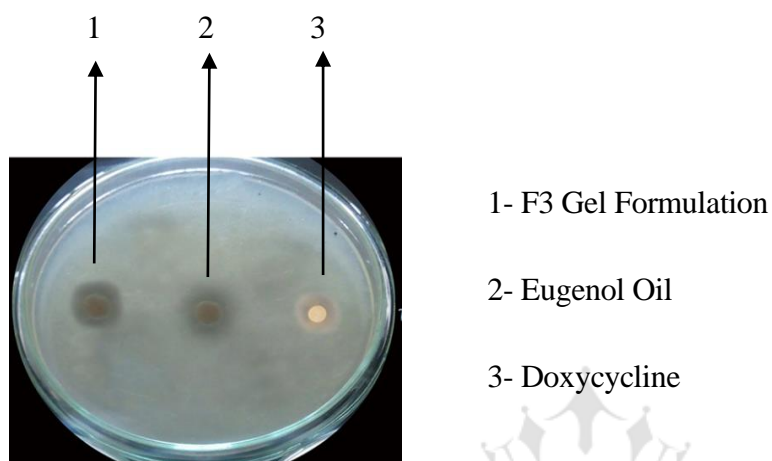


Figure No. 5: Antimicrobial activity on S. salivarius



Figure No. 6: Antimicrobial activity of Eugenol Oil Gel Formulation on S. salivarius

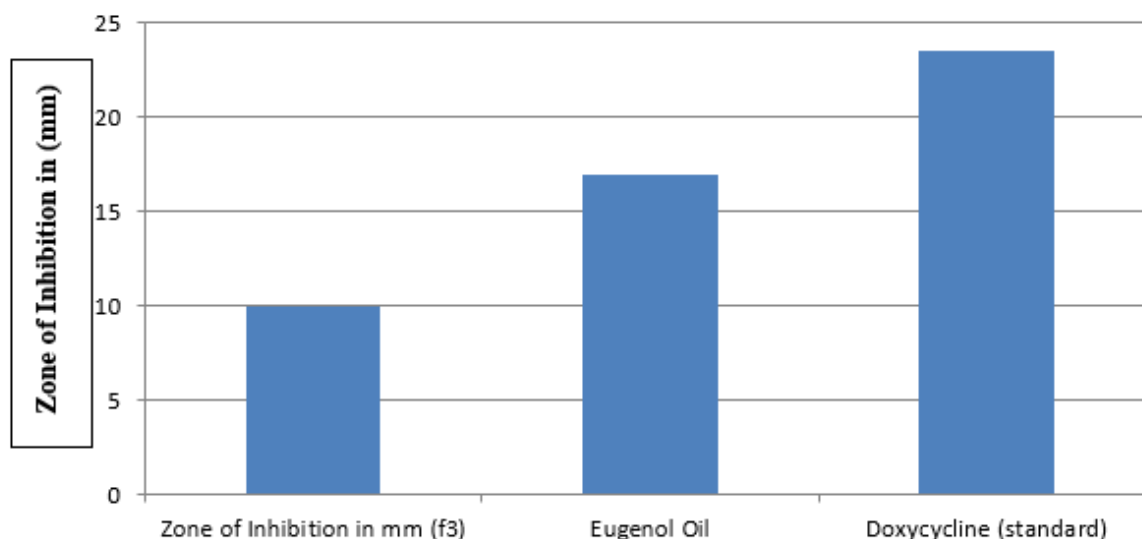


Figure No. 7: Antimicrobial activity on S. salivarius

Table No. 5: characteristics of gel formulations

Formulations	Appearance	pH	Spreadability (g-cm/sec)	Extrudability %	Homogeneity	Drug% content
F1	Pale yellow	6.6	18.20	92.14	Good	95.00
F2	Pale yellow	6.7	18.14	93.15	Good	95.20
F3	Pale yellow	6.7	17.49	94.10	Very Good	97.53
F4	Pale yellow	6.6	16.72	90.23	Good	93.62
F5	Pale yellow	6.4	15.59	89.10	Very Good	89.80

DISCUSSION

The procured Eugenol Oil was characterized for the following parameters:

- Acid value: 3.84
- Ester value: 37.21
- Solubility: 100% soluble in ethanol.
- Density: 1.06 gm/ml.
- Saponification Value: 42.07

➤ Refractive index: 1.53

The formulations were developed by using Eugenol Oil of same concentration and carbopol 934 at different concentrations. Formulation composition is given in Table-1. All five batches of formulations were evaluated for physical properties. All the formulations were pale yellow and had characteristic odor of Eugenol Oil. The pH of all formulations ranged from 6.4-6.7, which was well within the normal pH range of buccal cavity 6-7, which substantiates that the prepared gels will be irritation-free. Stability study of the developed formulation was performed according to International Conference on Harmonization (ICH) guideline and stability data of F3 indicate that optimized formulation exhibit good stability behavior regarding pH 6.7 and Percentage drug content (97.53 %).

As the formulation of Eugenol oil gel showed action against various pathogens that causes gum problem. The formulated Eugenol Gel exhibited good activity as compare to other drugs which are used against gum disease. The formulated gel shows excellent zone of inhibition, therefore, it may be concluded that formulated Eugenol gel has the potential to exhibit antimicrobial activity.

CONCLUSION

The Eugenol Oil was found to have antimicrobial activity against *Streptococcus salivarius*, *Streptococcus sanguis*, *Lactobacilli acidophilus*. The formulations developed from clove showed important results thus it may be more used commercially to develop dental gels when conducting clinical trials on masses. Nevertheless, further research is still needed to determine if they efficiently could substitute synthetic antibiotics or uses in combinations.

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