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FORMULATION AND EVALUATION OF DENTAL ANTI CARIES GEL BY *EUGENIA CAROPHYLLUS*

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ABSTRACT

The aim of the research is to formulate dental gel utilizing extract Clove. In multiple clinical studies and used in dentistry for wound healing effects, gingivitis, plaque control and. Clove is a natural, ancient ingredient. The formulated Clove tooth gel was evaluated by physical examination: Colour Crimson dark brown, Appearance-Homogeneous, Smooth nature, Transparency-Translucent, No microbial growth in sample plate, pH-7.02, Viscosity-322cp, spreadability-216.9 gm.cm/s and observed good stability. The anti-microbial evaluation against S. aureus reveals that the formulated Clove tooth gel exhibited notable activity with a ZOI of 18 mm at MIC of 25µg/mL. Herbal anti-caries gel has an anti-biofilm activity as compared to non–herbal drugs. Clove extract containing gel ensures no reinfection due to dental calculus. Also non – herbal formulations bring some side effects like an increase in the staining of teeth, alteration in taste perception, and an increase in calculus formation.

KEYWORDS: Clove, Dental Gel, Spreadability, pH, Herbal, Evaluation.

INTRODUCTION

What are the Teeth? They are the white-colored dense and hard projections coming out of our gums. The tooth is the strongest thing is the human body. Teeth are an important part of our oral cavity and play an important role in cutting and grinding food particles. Structure of Tooth Human teeth are composed of four tissues, the soft tissue of the Pulp and the three calcified tissues Dentin, Enamel, and Cementum. The cementum, also called root cementum, is simultaneously a part of the periodontium, the supporting structure of the teeth. The dental pulp occupies the pulp cavity of the teeth. They can be further divided into coronal pulp, which occupies the pulp chamber, and radicular pulp, which fills the pulp or root canals. The pulp contains cells (Odontoblasts, Fibroblasts, undifferentiated mesenchymal cells), nerve fibers, blood, and lymph vessels. The pulp cavity is lined by a layer of cells called Odontoblasts. Dentinal tubes give dentine its characteristic structure. chemically, dentin is composed of, by weight, 70% inorganic (mainly calcium and phosphate in hydroxyapatite crystals), 20% organic matrix (mainly collagen), and 10% water. Dentinal tubules contain the odontoblasts processes (Tome's Processes). Plaque is a mixture of bacteria, minerals, and some food residue. Some plaque hardens into calculus. Often, plaque and calculus do not come off with the brush and floss and cause inflammation, an ongoing state of gingivitis. Various preventive and treatment modalities are used in this perspective which includes oral hygiene, gingival irrigations and mechanical therapies like manual scaling and root planning, ultrasonic scalars and lasers. Mechanical instrumentation, full mouth disinfection, host modulation, and antimicrobial therapy are used in the non – surgical management of periodontal infections. Undesired effects of antimicrobial chemicals and antibiotics create the interest of dental product manufacturers towards herbal drugs to avoid side effects.[1]

Clove consists of dried flower buds of Eugenia caryophyllus, family Myrtaceae.

Morphological Characters :Colour : Crimson to dark brown, Odour : Slightly aromatic, Taste : Pungent and aromatic, followed by numbness, Size : About 10 to 17.5 mm in length, 4 mm in width, and 2 mm thick, Shape : Hypanthium is surmounted with 4 thick acute divergent sepals.[2]

What is Herbal Drugs / Medicines?

Herbal drugs also called botanical drugs or phytomedicines refer to the use of any plants, seeds, berries, roots, rhizomes, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more mainstream as up-to-date analysis and research show its value in the treatment and prevention of disease.

Herbal products themselves are stable in nature so it don't require stabilizers of preservatives. Also use of sodium benzoate-like preservatives in non–herbal dental

product may promote the formation of more cavities. Herbal dosage forms are very dilute in nature, so they reduce the possibility of allergic reactions. Herbal dental caries gel possesses antimicrobial activity as well as it also has Anti-biofilm activity i.e. it reduces the adhesion of bacteria to tooth surface. Synthetic or Non – herbal anti-caries preparations are bitter in taste and left behind their aftertaste.[3]

MATERIAL AND METHOD

Chemicals: Carbopol-940, Sodium Carboxy Methyl Cellulose, Polyethylene Glycol-4000, Triethanolamine, Sodium Benzoate, Methyl Paraben, and Propyle paraben, Ethanol, Glycerin were purchased from the market.

Extraction

Several methods were used to prepare clove extract for example Steam hydro distillation Soxhlation, Maceration. Out of the this maceration given best results so the method for the same is given below with quantities of ingredients taken for it[4]:

1) Clove powder – 30 gm, 2) Ethyl alcohol – 10 ml, 3) Distilled water – 300 ml

Procedure: The above-mentioned quantities of crude drug and solvents are taken, and mix together in a beaker. The beaker was wrapped in filter paper on which a petri plate was kept. And the beaker was kept a side for 5 days. After 5 days the beaker was shaken and mixture is subjected for filtration. After the formation of mark it was strained a couple of times with distilled water. Filtrate formed after filtration is then added in a Separatory funnel, 100 ml of Chloroform is added in it, then the lid was closed and the separatory funnel was shaken gently for 2 minutes and allowed to stand until two different phase are formed. (For obtaining clove oil from aqueous filtrate organic solvent is added (chloroform), due to partition coefficient contents of clove oil which are more soluble/miscible in organic solvent distribute itself in the organic layer). After 30 minutes when two layers are formed and the organic layer turned yellowish to brownish in color, then the organic layer is taken out in a beaker. The beaker was then wrapped by filter paper and kept aside overnight. (Beaker was kept aside overnight so that the chloroform would get evaporated and clove oil left behind).[5]

Formulation

Formulation of Dental Gel: Two different kinds of approaches were followed to formulate dental gel. The first approach was concerned with the use of Carbopol 934 as a gelling agent. Whereas in the second approach it was formulated by using Sodium Carboxy Methyl Cellulose (Sodium CMC).

1) First approach – Novel Type of Gel Formulation.

Sr. No.	Ingredients	Application	NG 1	NG 2	NG 3
1)	*Eugenia caryophyllus* Extract	API		2 ml	2 ml
2)	Carbopol 934	Gelling Agent	0.4 gm	0.6 gm	0.8 gm
3)	PEG 400	Stabilizer	5 ml	5 ml	5 ml
4)	Methyl Paraben	Preservative	0.18 gm	0.18 gm	0.18 gm
5)	Propyl Paraben	Preservative	0.02 gm	0.02 gm	0.02 gm
6)	Ethanol	5 ml	5 ml	5 ml	5 ml
7)	Triethanolamine	pH Adjuster	q.s.	q.s.	q.s.
8)	Distilled Water	Vehicle	100 ml	100 ml	100 ml

NG : Novel Gel

Due to less practice of formulating such formulations, all formulations of Novel gel were found of poor quality for further studies. As a result second approach was taken in consideration.

2) Second Approach – Conventional Mucoadhesive Gel

Sr. No.	Ingredients	Application	F1	F2	F3
1)	*Eugenia caryophyllus* Extract	API	1.5 ml	1.5 ml	1.5 ml
2)	Sodium Carboxy Methyl Cellulose	Gelling Agent	1.25 gm	2.5 gm	5 gm
3)	Glycerine	Stabilizer	5 ml	5 ml	5 ml
4)	Sweetener	Flavouring Agent	0.70 gm	0.70 gm	0.70 gm
5)	Distilled Water	Vehicle	25 ml	25 ml	25 ml

Process of Formulation for Conventional Mucoadhesive Gel

Required quantities of Sodium Carboxy Methyl Cellulose and Distilled water are taken. Then both Sodium carboxy Methyl Cellulose and distilled water are mixed and stirred so that a gel base could form. Then it is kept aside for 15 minutes. On other hand 1.5 ml clove extract is added in 5 ml glycerin. Now the gel base is subjected to stirring at 100 rpm for 15 minutes, during this step sweetener is also added in gel base. Now the mixture of glycerin and clove extract is added drop wise in the gel base, with continuous stirring until homogenous gel is formed.[6][7]

Evaluation study

Evaluation parameters for Dental Gel

A) Formulation related Evaluation studies

All prepared gel formulations were subjected to evaluation using parameters like physical appearance, pH, viscosity, spreadability, stability study, Chromatographic analysis using TLC method.

Physical appearance of formulation

The gel formulations were visually inspected for colour, odour, consistency, grittiness, uniformity stickiness and homogeneity.[8]

pH measurement

The pH of prepared gels was determined using a digital pH meter. The pH meter was calibrated before each use with standard pH 4 and pH 7 buffer solutions.[8]

Viscosity

Viscosity of the prepared gels was measured by a Brookfield viscometer at 100 rpm, using spindle number 6. Viscosities were recorded at room temperature.[8]

Spreadability

Spreadability is an important property of dental formulation for patient compliance. About 0.5 gm of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 100 gm was allowed to rest on the upper glass plate. The increase in the diameter due to the spreading of the gels was noted.[9]

Syringeability

Treatment of severe cases requires administration of the drug directly into periodontal pocket by using an injectable system fast relief. In this view, syringeability of gel formulations was evaluated though 21 G needle.[8][9]

Stability Study using Cooling and heating test

The stability of the formulation was studies against temperature changes, after 48 hours of preparation. Gel formulation was placed at 45℃ for 48 hours and then 48 hours at 4℃ for three cycles. At the end of stability study, the samples were analyzed for their physical appearance as colour, pH, and viscosity.

Chromatographic method of analysis

The clove extract obtained is subjected to Thin Layer Chromatography(TLC), for separating compounds and to observe presence of different compounds in the extract. Selection of solvent system is a important part of TLC. Ethanol is used as a solvent in this test.

From literature review it was clear that terpenoid called Eugenol present in clove is mainly responsible for anti bacterial and anti biofilm activity. As per spot separation in TLC plate the RF value of eugenol was calculated by formula,

RF Value = Distance travelled by Solute / Distance travelled by Solvent, For TLC, the solvent system used was Benzene: Chloroform in the ratio of 7:3

The detecting Reagent used was Iodine Vapours.

Stepwise Procedure for TLC: TLC Plate (rectangular) was washed with Acetone and kept in hot air oven for 30 minutes at 100℃, to activate the plate. After activation of plate a very fine line was drawn on the paper at one end, simultaneously 14 ml Benzene and 6 ml Chloroform are taken in a 500 ml beaker and shaken well and a petri plate was placed over it. Spots of Standard clove oil and Formulation 2 was given on TLC plate with the help of capillary on the line. Then the TLC plate was places in the beaker containing solvent system in such a way that, solvent system level remain below the spots. And solvent system is allowed to develop on the plate and then the lid was closed. Once the solvent reached particular height then the plate was taken out and dried. Then in a beaker Iodine water was taken and it was heated on burner and allowed to boil. Once the vapours started coming then plate was allowed to come in contact with the Iodine vapours. After a minute the plate was taken aside. The plate was observed under U.V. lamp in short wavelengths. Following are the images of TLC plate after completion of above steps.[10]

Fig 4. TLC Plate in Short UV Wavelengths.

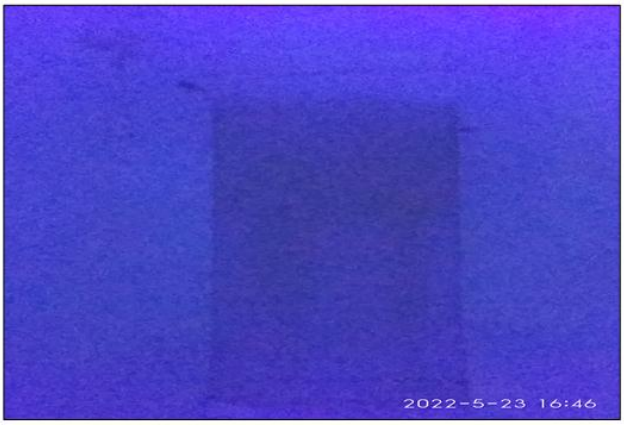

Fig 5. TLC Plates in Long UV Wavelengths.

Fig 6. TLC Plates in Day light.

RF Value = Distance travelled by solute / Distance travelled by solvent.

Anti – Microbial Activity Test - This test is of great importance for this formulation and its intended activity. Nutrient agar was used as media for microbiological study, Disc Diffusion Method was used to test the anti-microbial activity of formulation against *Staphylococcus aureus*. Nutrient Agar – Stepwise procedure – Required quantity of nutrient agar was taken in a conical flask, required amount of distilled water was added and the mixture is subjected to heating until it get boiled. After the nutrient agar boil at once then it was subjected to autoclaving at 121℃ and 15 lbs pressure for 15 minutes. After sterilization of nutrient agar, a set of petri plates was taken and sterilized. After sterilization of petri plates the sterilized nutrient agar was poured in the plates and allowed to cool down until it get solidified. Using aseptic techniques S. aureus was inoculated on the nutrient agar of petri plate using streaking method. Then the petri plate was kept in incubator for 24 hours at 34℃, so that the microbes could grow. After 24 hours growth, petri plates was taken out from incubator. 4 holes was made aseptically in nutrient agar using cork borer. And abbreviations – F1, F2, F3, Standard oil was made on outer glass surface. The holes were filled with F1, F2, F3, Standard oil as per their mark. Then the lid was closed and again the petri plates were kept in incubator of 24 hours at 34℃.After 24 hours of formulation application the petri plates were taken out and subjected to calculations. Following are the results obtained after the anti – microbial study of gel formulations.[11][12]

Formula to calculate diameter of zone of inhibition:

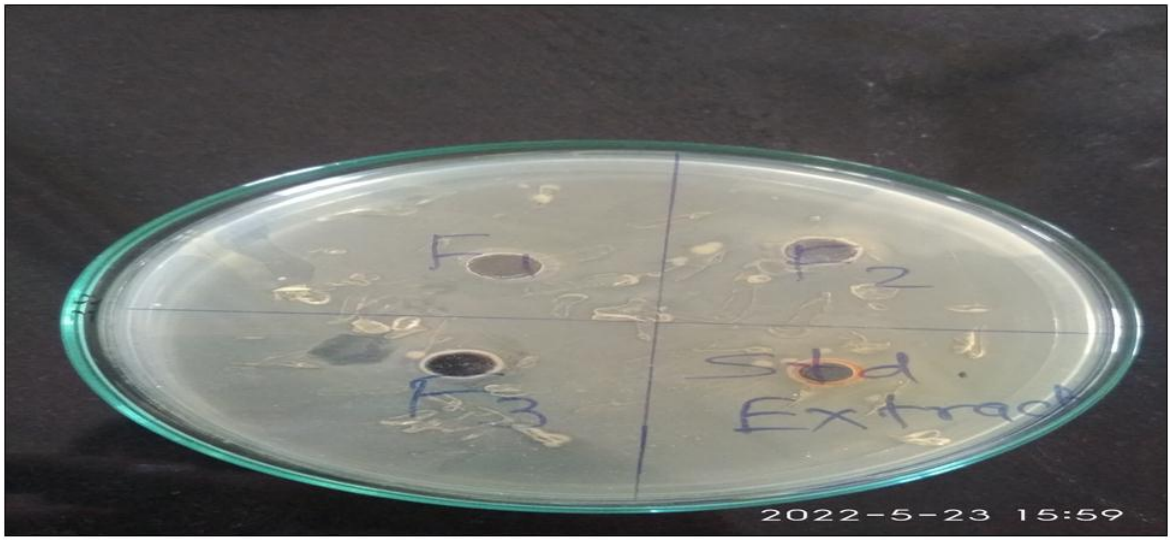

Fig 7. Petri plate after 24 hours contact time with formulations and standard oil.

C) Crude Drug related Evaluation studies

a) Morphological / Organoleptic Tests

1) Colour – Dark brown
2) Odour – Aromatic
3) Taste – Pungent

b) Microscopic Study

i) Transverse Section of Crude Drug

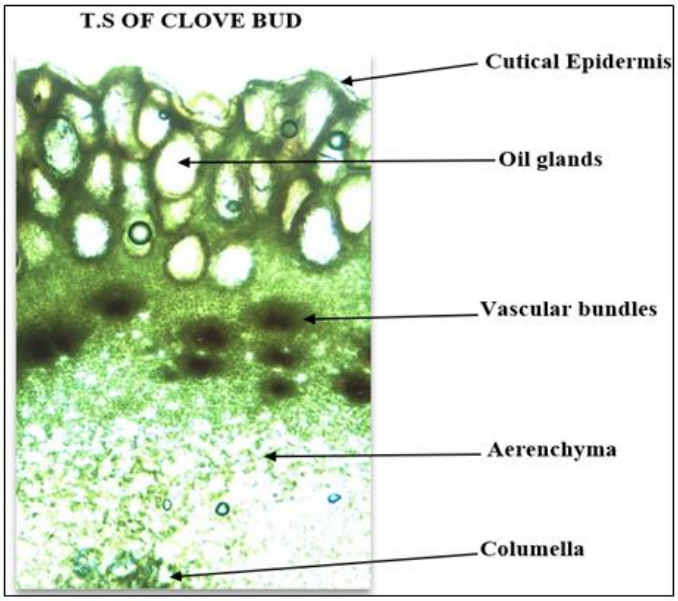

Fig 8.

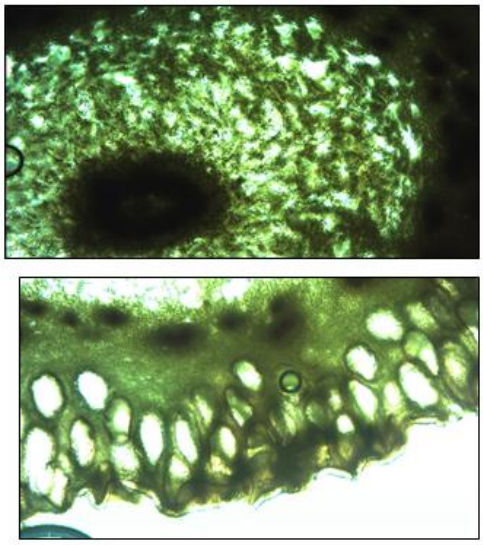

Fig. 10

Description – The transverse section of clove bud clearly shows thick outer layer of Cutical Epidermis. Below that there are many Oil glands. Below the layer of Oil glands there are dark coloured vascular bundles. Beneath the vascular bundles there is a layer of Aerenchyma followed by layer of Columella.[13]

Powdered Microscopy

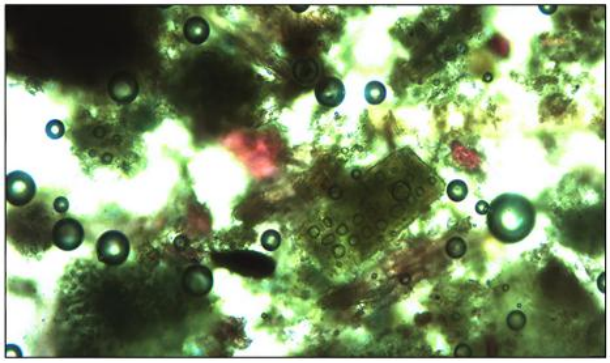

Fig. 11

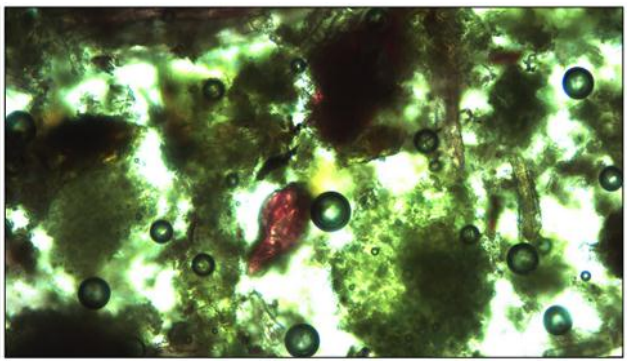

Fig. 12

Fig. 11, 12 = Powder Microscopy of Clove bud.

Powdered microscopy of Clove powder shows epidermal cells, phloem fibers, sclereid cells and fragment of hypanthium showing a portion of the oil gland.

RESULT

a) Physical Appearance

Sr. No.	Property	Inference
1)	Colour	Shiny light yellow
2)	Odour	Sweet aromatic
3)	Spreadability	Good
4)	Homogeneity	Homogenous
5)	Syringeability	Good

b) Phytochemical Tests

Sr. No.	Phytochemicals	Inference
1)	Alkaloids	Present
2)	Steroids	Present
3)	Tannins	Present
4)	Flavonoid	-
5)	Terpenoids	Present
6)	Glycosides	Present
7)	Phenolic Compounds	Present

(- sign indicates absence of phytochemical or negative result after testing)

a) Physical Constant Tests

Sr. No.	Test	Value
1)	Ash Value	
2)	Acid Value	3.843
3)	Saponification Value	42.07
4)	Ester Value	38.22

b) Calculations of RF Value

From all 3 formulations F2 shown optimum activity so it was chosen for TLC. The calculations for retention factor are given below

RF Value of F2 :

Distance travelled by solute – 4 cm

Distance travelled by solvent – 6.3 cm

Formula = Distance travelled by solute / Distance travelled by solvent

= 4 / 6.3 = 0.6349

F2 = RF Value = 0.6349

For Standard Clove Oil

Distance travelled by solute = 4.8 cm

Distance travelled by solvent = 6.3 cm

RF Value = 4.8 / 6.3 = 0.7619

Sr. No.	Samples	RF Value
1)	Formulation 2	0.6349
2)	Standard Clove Oil	0.7619

c) Anti - Microbial Activity

Sr. No.	Samples	Diameter of Zone of Inhibition (mm)
1)	Formulation 1	14
2)	Formulation 2	18
3)	Formulation 3	23
4)	Standard Clove Oil	10

d) Physical Tests of Formulation

i) Spreadability Test

Sr. No.	Formulation	Spreadability (gm.cm/s)	Time (seconds)
1)	F1	215.7	18.4
2)	F2	216.3	17.3
3)	F3	213.9	18.1

ii) Viscosity

The viscosity was recorded at 37℃ using Brookfield Viscometer.

Sr. No.	Formulation	Viscosity (cps)
1)	F1	320
2)	F2	322
3)	F3	316

iii) pH Value

Sr. No.	Formulation	pH Value
1)	F1	6.88
2)	F2	6.95
3)	F3	7.02

DISCUSSION

All formulation prepared were subjected to tests like spreadability, syringeability, viscosity, stability test, anti-microbial activity, etc. F2 is the formulation which shows optimum performance across all tests. F2 contain 1.5 ml clove extract and at this concentration its pH was 6.95 and thus it is compatible with oral tissues. Also it has good viscosity, stability, spreadability, and syringeability so we conclude that Formulation No. 2 will be used for future studies.

SUMMERY AND CONCLUSION

Sodium Carboxy Methyl Cellulose is added in the formulation to ensure slow but continuous release of phytochemicals. So that long duration of action could be achieved. Whereas glycerin promotes gel like consistency and prevent the hardening of gel. Saccharin Sodium masks the taste of clove extract and makes the formulation more acceptable. Herbal anti caries gel has a anti-biofilm activity as compared to non – herbal drugs. Clove extract containing gel ensures no reinfection due to dental calculus. Also non – herbal formulations bring some side effects like an increase in the staining of teeth, alteration in taste perception, an increase in calculus formation. The main ingredient i.e. clove extract has dual activity bactericidal effect and anti biofilm. This means it not just kill the infecting bacteria as well as it prevent from entering in a dormant phase. The herbal anti caries formulation is easy to apply, easy to wash, and it is convenient to patient. Though the anti microbial activity has shown promising results further study is to be done in future.

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