



FORMULATION AND EVALUATION OF DENTAL GEL CONTAINING CLOVE OIL FOR THE TREATMENT OF PERIODONTAL DISEASES

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ABSTRACT:

The main objective of this work is the formulation and development of herbal products such as dental gel of clove oil for the treatment of periodontal disease. Periodontal disease is recognized as a one of major public health problem throughout the world and occurs in all groups, ethnicities, races, genders and socio-economic levels. The active ingredient of this dental gel is clove oil. The clove oil was evaluated for physical parameters like acid value, ester value, saponification value and specific gravity. The prepared gel was evaluated for various properties such as 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 periodontitis. Clove oil showed the zone of inhibition of about 22.05 ± 0.04 mm. On the basis of the result obtained in this present study we conclude that the gel formulations of clove oil F3 showed good physicochemical properties as well as good drug content compared to other formulations.

KEY WORDS: Clove oil, Carbopol 934, Periodontitis, Anti microbial activity.

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 periodontitis. Gingivitis, the moderate stage of disease caused by an accumulation of supragingival plaque and characterized by swelling. Light bleeding and redness of the marginal gingival. Gingivitis is associated with a change in the microflora, shifting from a Gram-positive anaerobic flora to a more Gram negative one. Periodontitis, a more severe stage of periodontal disease, result in the resorption of the alveolar bone and detachment of periodontal ligament support tooth ^[2]. Recent advances in the field of dentistry have promoted the use of herbal and natural products for the treatment of various oral diseases. 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]. Clove oil is one such product exhibiting multiple benefits and has gained considerable importance in clinical research^[4]. Since clove 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 clove oil for the treatment of periodontal diseases and then evaluated for their physicochemical properties including drug content, spreadability, extrude ability and in-vitro antibacterial activity.

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine.

Benefits of herbal drugs:

- Herbal drugs have long era of use and better patient tolerance as well as public acceptance.
- Herbal drugs acts as a renewable source, which is our only hope for population.
- The cultivation and processing of medicinal herbs and herbal products is environment-friendly.
- Throughout the world, herbal medicine has provided many of the most useful and vast variety of drugs to the modern medical science.

Need of investigation:

The reason behind doing this investigation is to prepare best therapy for periodontal disease. Periodontal disease is recognized as a major public health problem throughout the world and occurs in all groups, ethnicities, races, genders and socioeconomic level. Treatment for periodontal disease is needed but some of the drugs will be cost effective so we need to use another type of drug therapy. Herbal plants used traditionally for number of diseases. Herbal will cure the root of disease. Study on clove plant which is also called as *Syzygium aromaticum* plant is needed to do investigate this plant. In North India clove plant buds used which control periodontal disease. So we decided to make formulation on clove plant. So herbal formulation will be useful for number of people affected by periodontal disease.

Dental gel of clove oil is used for the treatment of periodontal disease. This gel is for pain relief of gums. Clove oil is one such product exhibiting multiple benefits and has gained considerable importance in clinical research. The active ingredient of this dental gel is clove oil. Clove is the strongest pain relieving agent. Clove oil contains the main ingredient of eugenol, which is a natural anesthetic. Since clove 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.

Aim&Objective:

The study was aimed to develop and evaluate dental gel containing clove oil as the chief constituent for the treatment of periodontitis.

- To perform clove oil characterization.
- To formulate dental gel of clove oil using gelling agent and other ingredients.

Plan of work:

- Exhaustive literature survey
- Selection of suitable drug and excipients
- Procurements of materials

Preformulation study of the pure drug

- Morphology activity
- Microscopic characteristic
- Identification test
- Physicochemical characteristics of pure drug (clove)

Experimental study

- Formulation and development of dental gel containing clove oil for the treatment of human periodontal disease.
- Optimize the formulation by performing the evaluation test of Gel formulation
- Antimicrobial activity
- Stability study

Plant Profile¹⁰:



Figure No.1. Clove Plant

Synonym: Caryophyllum; clove flower, clove bud; launge.

Biological source:

Cloves consists of dried flower buds of *Eugenia caryophyllus* (Myrtaceae). It should contain not less than 15% (v/v) of clove oil.

Chemical constituents:

15-20% of volatile oil; 10-13% of tannin(gallotannic acid), chromone and eugenin. The volatile oil contains eugenol (about 70 to90%), eugenol acetate, methylamylketone.caryophyllenes and small quantities of ester and alcohols. Some Important components are; Flavonoids, Hidroxibenzoic acid,Hidroxicinamic acid, Hidroxiphenyl propens, Eugenol, Phenolic acids, Gallic acid, Kaempferol, Quercetin, Caffeic acid, Ferulic acid, Elagiacid, Salicylic acids. B-cariofileno.Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of freshplant material.

Uses:

- 1) It is used as a Dental analgesic.
- 2) It is used as a Carminative.
- 3) It is used as a Stimulant.

Scientific classification of clove:**Figure No.2.Clove Bud****Classification:**

| | |
|----------------|----------------|
| Kingdom | Plantae |
| (Unranked) | Angiosperms |
| (Unranked) | Eudicots |
| (Unranked) | Rosids |
| Order | Myrtales |
| Family | Myrtaceae |
| Genus | Syzygium |
| Species | S. aromaticum |

Table No.1.Scientific classification of clove**Biological Activity:**

Clove is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries and reported in literature. A review of several scientific reports of the most important biological activities of clove and eugenol is presented in the following paragraphs.

Antioxidant Activity:

Recently, the United States Department of Agriculture in collaboration with Universities and private companies create a database with the polyphenol content and antioxidant activity of different kind of foods. Among spices, clove showed the higher content of polyphenols and antioxidant compounds. Extracts from clove buds could also be used as food antioxidants. The shelf-life and frying stability of encapsulated and un-encapsulated eugenol-rich clove extracts were tested soybean oil. Controlled release of antioxidants could be achieved by encapsulated clove powder obtained by spray drying using maltodextrin and Arabic gum as wall materials.

Antimicrobial Activity:

The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Sofia et al. tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested Escherichia coli (E. coli), Staphylococcus aureus and Bacillus cereus was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

Morphology Studies^[12]:-

Microscopical characters of Clove flower bud:

Transverse section of clove hypanthium below the ovary shows epidermis, cortex and columella:

1) Epidermis:

Single layered small cells with straight walls and has a very thick cuticle. Epidermal layer gets intercepted by Ranunculaceous type of stomata.

2) Cortex:

The three distinct zones or regions in the cortex can be made out. The peripheral region containing 2 to 3 layers of big ellipsoidal, schizo-lysigenous oil glands embedded in the radially elongated parenchymatous cell. The middle region containing 1 or 2 rings of bicollateral vascular bundles associated with a few pericyclic fibres, embedded in thick walled parenchyma. The inner region made of loosely arranged aerenchyma.

3) Cork and primary cortex- absent

4) Stellar part - phloem, phloem fibres, biseriate medullary rays and secretory cavities containing volatile oil.

5) Cortical parenchyma - starch grains

6) Columella:

Forms the central cylinder containing thick wall parenchyma with a ring of bicollateral vascular bundles towards the periphery of the cylinder. Numerous sphaeraphides are seen scattered throughout the columella and to a certain extent in the middle cortical zone.

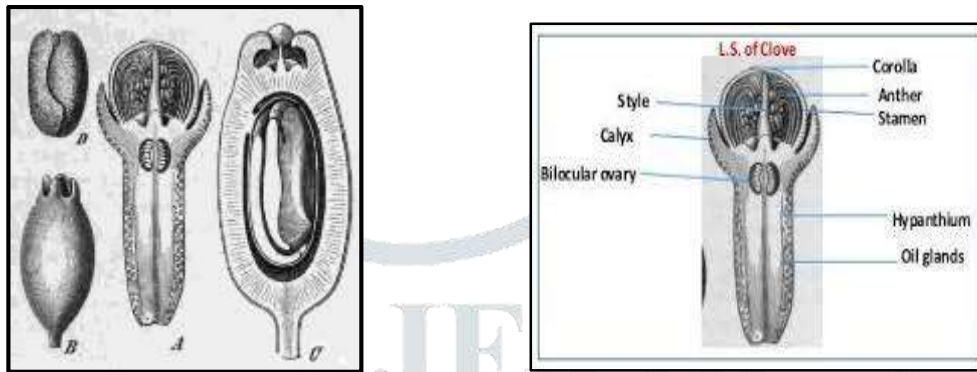


Figure No.3(a). L.S. of Clove

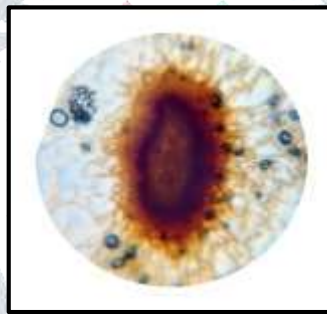


Figure No.3(b). T.S. of Clove

Macroscopical characters:

The volatile oil is situated in the schizolysigenous oil glands or ducts which are present in all 1 parts of the flower buds (hypodermis).

1) **Size:** Length varies from 12 to 17 mm.

2) **Type:** Actinomorphic, bisexual, epigynous. The flower bud has a spherical head and a sub-cylindrical hypanthium tapering at the lower end.

3) **Calyx:** Polysepalous, 4 hard and thick sepals with oil glands.

4) **Corolla:** Polypetalous, 4 petals imbricate, enclose the stamens and forms the head of the bud Androecium numerous stamens, free and introrsely.

5) **Gynasium:** Binocular, inferior with ovules stamens, free Placentation axial.

6)Style: Single and erect.

7)Colour: Dark brown.

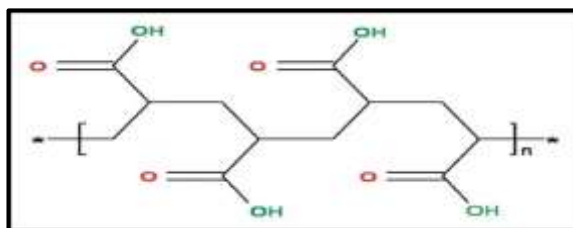
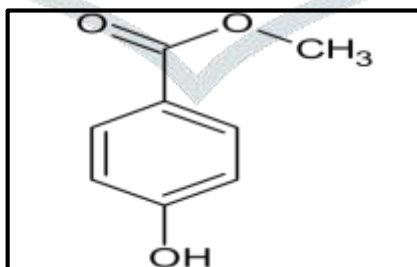
8)Odour: Aromatic, spicy, Strong.

9)Taste: Pungent, aromatic.

Identification test of clove oil^[14]:

| Sr.No. | Test | Observations |
|--------|--|--|
| 1 | Thick section +50% of potassium hydroxide solution | Needle shaped crystals of potassium eugenate. |
| 2 | A drop of ferric chloride solution previously dissolved in 5ml of alcohol + a drop of clove oil | Blue colour is produced due to phenolic –OH group. |
| 3 | A drop of chloroform extract of clove + a drop of 30% aqueous solution of sodium hydroxide saturated with sodium bromide | Needle and pear shaped of crystals of sodium eugenate. |

Table No.2.Indentification test of clove oil

Excipient profile:**1)Carbapol-****Structure:****Figure No.4. Structure of Carbapol****IUPAC Name:** Poly(acrylic acid)**Other Names:** PAA, PAAC, Acrysol, Acumer.**Chemical Formula:** (C₃H₄O₂)**Molar Mass:** variable**USES:** Polyacrylic acid and its derivatives are used in disposable diapers ion exchange resins and adhesives. They are also popular as thickening, dispersing suspending and emulsifying agents in pharmaceuticals.**2) Methyl Paraben-****Structure:****Figure No.5. Structure of Methyl Paraben****IUPAC Name:** Methyl 4hydroxybenzoate**Other Names:** Methyl Paraben**Chemical Formula:** C₈H₈O₃

Molar Mass: 152.15g mol⁻¹

USES: Methyl paraben is an antifungal agent often used in a variety of cosmetics and personal care products.

3) Polyethylene glycol-

Structure:

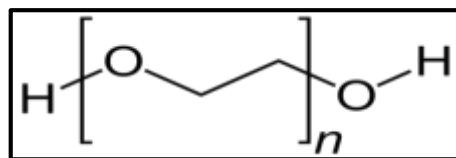


Figure No.6. Structure of Polyethylene Glycol

IUPAC Name: poly(ethylene)oxide

Other Names: Polyoxyethylene

Chemical formula: C_{2n}H_{4n+2}O_{n+1}

Molar mass: 44.05n+18.02g/mol

USES: Polyethylene glycol 3350 is used to treat occasional constipation. Polyethylene glycol 3350 is in a class of medications called osmotic laxatives. It works by causing water to be retained with the stool. This increases the number of bowel movements and softens the stool so it is easier to pass.

4) Propyl paraben-

Structure:

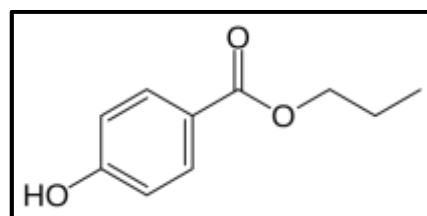


Figure No.7. Structure of Propyl Paraben

IUPAC Name:4-hydroxybenzoic acid

Other Name:n-propyl paraben,isopropylparaben

Chemical formula:C10H12O3

Molar Mass:180 gm/mol

USES:It is widely used as preservatives by pharmaceutical and cosmetics industry.They are effective.These compounds and their salts are used mainly for their antifungal and antibacterial properties.

Materials and Methods:

Materials: The dental gel of clove oil was prepared using following chemical apparatus and instrument

Chemicals: Clove oil, Carbapol, Polyethylene glycol, Glycerine, Methyl Paraben, Propyl Paraben , Honey Distilled water.

Apparatus: Apparatus such as beaker, glass slide, measuring cylinder, test tube, mortar pestle volumetric flask, sonicator apparatus.

Instruments: pH meter, Mechanical stirrer, Viscometer, Incubator,Auto-clave

Materials and its functions:

| Sr. No. | Materials | Functions |
|---------|---------------------|-----------------------------------|
| 1 | Clove oil | Active pharmaceutical ingredients |
| 2 | Carbapol 934 | Gelling agent |
| 3 | Polyethylene glycol | Co-solvent |
| 4 | Glycerine | Drug solubiliser |
| 5 | Methyl paraben | Preservatives |
| 6 | Propyl paraben | Preservatives |
| 7 | Honey | Sweetening agent |
| 8 | Distilled water | Vehicle |

Table No.3. Materials and its functions

Physico-chemical characteristics of clove oil:**1) Acid Value:**

The acid value is defined as the number of milligrams of Potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides.

Chemicals: Oil sample, phenolphthalin indicator, ethanol, sodium hydroxide

Apparatus: Burette, stand, conical flask, measuring cylinder

Formula for acid value: $(\text{Mol. wt} \times N \times V) / W_s$

2) Saponification Value:

Saponification value is defined as the number of milligrams of KOH required to neutralize the fatty acids resulting from complete hydrolysis of 1 gm of sample of oil or fat.

Chemicals: Oil sample, ethanol, phenolphthalin indicator, 0.1N KOH, 0.1N HCL.

Apparatus: Conical flask, pipette, burette, beaker, round bottom flask, water bath, reflux condenser.

Calculation and Observations:

Saponification value = mg of KOH consumed by 1 g clove oil

Weight of KOH = Normality of KOH \times Equivalent weight \times volume of KOH in litres

Volume of KOH consumed by 1 g of oil = [blank- test]

Formula for Saponification value = $((B-T) \times N \times 56.1) / W_t$ of oil

4) Ester value:

Ester value = Saponification value - Acid value Solubility

5) Density:

Density = Mass of oil / Volume of oil

Formulation of Dental gel of clove oil:**Composition of dental gel:**

| Sr.No. | Ingredients | F1 | F2 | F3 | F4 |
|--------|----------------------------|------|------|------|------|
| 1 | Clove oil(ml) | 0.75 | 0.75 | 0.75 | 0.75 |
| 2 | Carbapol 934 (gm) | 0.3 | 0.4 | 0.5 | 0.6 |
| 3 | Polyethylene glycol(ml) | 15 | 15 | 15 | 15 |
| 4 | Methyl paraben(gm) | 0.18 | 0.18 | 0.18 | 0.18 |
| 5 | Propyl paraben(gm) | 0.02 | 0.02 | 0.02 | 0.02 |
| 6 | Glycerine(ml) | 5 | 5 | 5 | 5 |
| 7 | Honey(ml) | 0.4 | 0.4 | 0.4 | 0.4 |
| 8 | Distilled water(ml) | q.s. | q.s. | q.s. | q.s. |

Table No.4. Composition of Dental Gel**Procedure for the preparation of dental gel:**

- 1) **Soaking** :soaked carbapol 934^[6] in water.
- 2) **Neutralization**:Neutralize with triethaloamine to pH 9.4.
- 3) **Addition of preservative**: Addition of propyl and methyl Paraben.
- 4) **Addition of co-solvent and API** : Addition of propylene glycol and clove oil in another test tube.
- 5) **Addition of sweetener**: Finally honey is added.
- 6) **Stirring**^[7]: Stirring is done until a homogeneous product is formed.

Evaluation Parameters^[17]:

- **Appearance:**

All the formulations of clove oil gel were pale yellow in colour .

- **Consistency:**

The consistency was checked by applying on skin.

- **Greasiness:**

The greasiness was assisted by the application on to the skin.

- **Determination of pH:**

pH of gel was determined using digital pH meter by dipping the glass electrode completely into the gel system.[9]

- **Determination of viscosity:**

Viscosities of the formulated gels was determined using Brooke field viscometer, spindle no. 7 and spindle speed 60 rpm at 25-C was used gels, the corresponding dial reading on the viscometer was noted[10].

- **Determination of spreadability:**

Spreadability was determined using following formula,

$$S=M.L/T$$

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 sign a tube filled with the gel having a tip of sim opening and by measuring the amount of gel that extruded through the tip when a pressure was applied on the tube was noted down.

- **Stability study:**

Physical stability study tests of the formulation was carried for one weeks at temperature of 37°C.the formulation was found to be physically stable at temperatures of 37°C. Within one weeks.

- **Antimicrobial Activity:**

The nutrient agar media was used.S.salivarius microorganism culture was used. Incubation time was setup for 24 hours.

S.salivarius suspension was introduced in each plates and 40ml of sterile agar media was poured into each sterilized plates. The plates were agitated carefully to allow homogeneous mixing of the agar with the plates were left on the flat solid. surface and allow to harden. In each plate 1 cup 10ml in diameter was bored in the medium with cork bore the disc of agar bore were removed by sterilised dissecting needle while being careful not be damage the cups. In each plate equal amount of gel Formulation having same strength was placed in the cup and we're incubator at $37^{\circ}\text{C} + 2^{\circ}\text{C}$ for 24 hrs in incubator the entire operation was carried out under aseptic condition and zone of inhibition was calculated the zone.

Zone Of Inhibition:

The antibacterial activity was performed by agar well diffusion method. The plates were evaluated after incubation at 37°C for 24 hours after which the zone of inhibition around each was measured by using scale in millimetres (mm). The ratio between the diameter of inhibition zone (mm) produced by plant extracts and the inhibition zone around the well with formulation was used to express antibacterial activity.

- **Comparative study of marketed preparation and herbal preparation of dental gel of clove oil:(spreadability)**

Result and discussion:

Identification test :



Figure No.8. Identification test of Clove Oil

A)Physicochemical characterstics of clove oil :

| Sr.No. | Parameters | Clove oil procured | Clove oil standard |
|---------------|-------------------|---------------------------|---------------------------|
| 1 | Colour | Pale yellow | Pale yellow |
| 2 | Odour | Aromatic | Aromatic |
| 3 | Acid value | 3.66 | 3.84 |
| 4 | Ester value | 37.21 | 38.22 |

| | | | |
|---|-----------------------|----------------|----------------|
| 5 | Solubility in ethanol | Freely soluble | Freely soluble |
| 6 | Density | 1.02g/ml | 1.05g/ml |

Table No.5 Physicochemical characteristics of Clove Oil



Figure No.9. Formulation of Dental Gel Of Clove Oil

B) Characteristics of gel formulation:

| 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 | 95.40 |
| F4 | Pale yellow | 6.6 | 16.72 | 90.23 | Good | 93.62 |

Table No.6.Characteristics of Gel Formulation

C) Stability study (Evaluation Test After One Week):

| Formula tions | Appearan ce | pH | Spreadabil ity(g- cm/sec) | Extrudabil ity% | Homoge nity | Drug conte nt |
|---------------|-------------|-----|---------------------------|-----------------|-------------|---------------|
| F1 | Pale yellow | 6.6 | 17.28 | 90.14 | Good | 94.00 |
| F2 | Pale yellow | 6.7 | 16.23 | 92.15 | Good | 94.20 |
| F3 | Pale yellow | 6.7 | 17.30 | 94.10 | Very good | 95.40 |
| F4 | Pale yellow | 6.6 | 15.30 | 91.23 | Good | 92.62 |

Table No.7.Stability Studies(After one week)

- Spreadability test of Dental Gel formulation:**

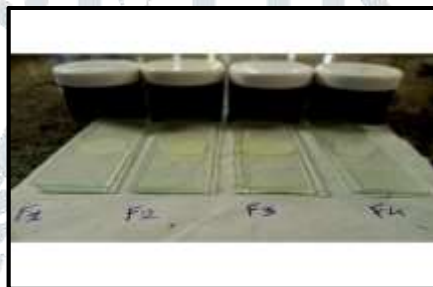


Figure No.10.Spreadability test of Dental Gel

- Antimicrobial activity on streptococcus salivarius.**



Figure No.11.Antimicrobial activity on streptococcus salivarius

| Microorganism | F3 | Clove oil |
|---------------|-------|-----------|
| S.salivarius | 22.05 | 23 |

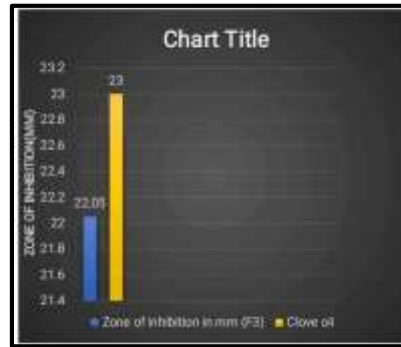


Figure No. 12. Graph Of Zone Of Inhibition

- comparative study:(spreadability)



Figure No.13. Comparative study of herbal and marketed preparation

The optimized batch of procured clove oil was characterized for the following parameters:

- Acid value: 3.66
- Ester value: 37.43
- Saponification value: 41.09
- Density: 0.93gm/mol

The formulations were developed by using clove oil of same concentration and carbopol 934 at different concentrations. All the formulations were pale yellow in colour and had characteristic odour of clove oil. The pH of all formulations range from 6.4-6.7, which was well within the normal pH range of buccal cavity 6-7. The spreadability of the gels was found to be in the range of 15.59-18.20 g-cm/sec, confirming that these gels may spread smoothly and uniformly. The formulations were glossy and translucent. The homogeneity and tube extrudability of all formulations was good. The drug content of the formulations was ranged from 89.8% - 95.40% Table-6. The formulation F3 was found to have maximum drug content. The gel formulations of clove

oil F3 showed good physicochemical properties as well as good drug content compared to other formulations . Hence, these formulations were further selected for anti microbial studies. The results of anti microbial studies showed that gel formulation of clove oil F3 showed a maximum zone of inhibition *s.salivarius*.

CONCLUSION:

The clove oil was found to have antimicrobial activity against *Streptococcus salivarius*. The formulations developed from clove showed significant results so it can be further used commercially to develop dental gels after conducting clinical trials on human beings. Nevertheless further research is still needed in order to determine if they efficiently could substitute the synthetic antibiotics or uses in combinations.

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