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FORMULATION AND EVALUATION OF DENTAL GEL CONTAINING CLOVE OIL FOR THE TREATMENT OF PERIODONTAL DISEASE- A REVIEW

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ABSTRACT

The study was aimed to formulate and evaluate dental gel containing clove oil as the chief constituent for the treatment of periodontitis. Clove oil has a wide spectrum of antibacterial activity against a number of periodontal pathogens, hence it is selected for the treatment of periodontitis. Clove oil gel is formulated by using carbopol 934 as gelling agent, clove oil as medicinal agent, polyethylene glycol as co-solvent, methyl paraben and propyl paraben as preservative and required quantity of distilled water as vehicle. 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, results in the resorption of the alveolar bone and detachment of the periodontal ligament supporting tooth. This study was aimed to formulate dental gel containing clove oil for the treatment of periodontal diseases and then was evaluated for their physicochemical properties including drug content, spreadability, extrude ability, in-vitro antibacterial activity.

KEYWORD: Dental gel, Clove oil, Antibacterial activity, Periodontal disease.

INTRODUCTION

Periodontal disease is recognized as a major public health issue throughout the world and occurs in all groups, ethnicities, races, genders and at 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.[16] Periodontitis is an inflammatory response to the overgrowth of anaerobic organisms such as *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus, Prevotella melaninogenica and Actinobacillus actinomycetem comitans.* The conventional method for treatment the periodontal disease like oral, topical and systemic dosage forms have the major disadvantages like superinfection, low or non-compliance, low gingival crevicular fluid levels of antibiotics, systemic side effects, short duration and high relative cost [18]. Periodontal treatment aims to cure inflamed tissue, reduce the number of pathogenic bacteria and eliminate the diseased 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 have been several reports on the use of traditional plants and natural products for the treatment of oral diseases. Clove oil is one such product exhibiting multiple benefits and has gained considerable importance in clinical research.[19] 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.

Periodontal Disease

Periodontal disease is considered as major public health problem throughout the world. Good daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can occur in all age groups, ethnicities, races and genders. Periodontal diseases including gingivitis and periodontitis are serious infections which can lead to tooth loss when left untreated. The word periodontal literally means "around the tooth." Periodontal diseases are infections of the bone

and gums that bound and support the teeth. In its early phase called gingivitis, gums can become inflamed and red, and they may bleed too in severe conditions. In its more severe form, called periodontitis, the gums can draw away from the tooth, bone can be lost, and the teeth may get loosen or even fall out.

It is a local infection with primary bacterial etiology in the gingival crevices, which affects the structural organs surrounding the teeth like periodontal ligament, connective tissue and bone. The warm and moist pocket environment fastens the growth of gram-negative, anaerobic bacteria that proliferate in the subgingival space. The most important and most prevalent anaerobic gram-negative bacteria in the subgingival area are *Actinobaci-llusactinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Tannerella forsythensis (Tf).*

Periodontitis, which is bacterially induced, can be defined as a" chronic inflammatory disease initiated by dental plaque biofilm and perpetuated by a week immune response (Suvan et al., 2011) usually accompanied by gingivitis resulting in irreversible destruction of the supporting tissues surrounding the tooth, including the alveolar bone".Therapeutic approaches for periodontitis fall into two major categories:

1. Anti-infective treatment, which is designed to stop the progression of periodontal attachment loss by removing etiologic factors; and
2. Regenerative therapy, which includes anti-infective treatment and is intended to restore structures destroyed by this disease. Essential to both treatment approaches is the inclusion of periodontal maintenance procedures. The main cause of periodontal diseases is bacterial plaque, a sticky, colorless film that constantly forms on your teeth. If the plaque is not removed, it can turn into a hard substance called calculus or tartar in less than two days. Tartar is so hard it can only be removed by an oral health professional, such as a dentist or dental hygienist. The bacteria in plaque infects the gums, and release poisons that cause redness and inflammation (irritation). The inflammation and the poisons themselves cause destruction to the tissues that support the teeth, including the bone. When this happens, the gums separates microscopically from the teeth, forming pockets that will eventually fill with more plaque causing even more infection.[1]

Etiology of Periodontitis

The undetectable, sticky film called plaque mainly composed of bacteria stays on the teeth for more than two or three days, can harden under the gum line into tartar (calculus). Tartar makes very difficult to remove plaque and acts as bacterial reservoir. Longer the plaque and tartar remains on the teeth, they cause irritation and inflammation of gingiva, which leads to pocket development between the gums and teeth. The pockets deepens and more bacteria builds up, which causes infection and eventually leads to loss of tissue and bone.[1]

Types of Periodontitis

1. Mild periodontitis (gingivitis)
2. Moderate periodontitis
3. Advanced periodontitis
4. Refractory periodontitis

Common Pathogens of Periodontitis

1. T.forsynthia
2. P.intermedia
3. F.nucleatum
4. A.actinomycetocomitans

Microbes and Their Specific Disease

Table 1.1: Microbes responsible for specific diseases.

Micro organism	Disease
Actinomyces viscosus some streptococci	Adult gingivitis
Porphyromonas gingivalis Haemophilus sp. Campylobacter rectus Fusobacteria sp. Selenomonas sputigena	Adult periodontitis
Eikenella corrodens	Rapidly progressive adult periodontitis
Intermediate spirochetes Fusobacteria	Acute necrotizing ulcerative periodontitis
Prevotella intermedia	Acute necrotizing ulcerative periodontitis Pregnancy gingivitis
Capnocytophaga sp.	Juvenile iabetes Immunocompromised Neutropenia
Actinobacillus Actinomycetemcomitans	Localized juvenile periodontitis

Signs and Symptoms

- Red, swollen or tender gums
- Gums that bleed when brushing or flossing
- Receding gums
- Deep pockets (the space between the gums and the teeth)
- Metallic taste
- Tooth sensitivity for no apparent reason
- Loose or shifting teeth
- Abscesses
- Pus around gums and teeth
- Chronic bad breath

Diagnosis

At a dental visit, a dentist or dental hygienist will:

1. Examine your gums and note any signs of inflammation.
2. Use a tiny ruler called a "probe" to check for and measure any pockets around the teeth. In a healthy mouth, the depth of these pockets is usually between 1 and 3 millimeters. This test for pocket depth is usually painless.
3. Ask about your medical history to identify conditions or risk factors (such as smoking or diabetes) that may contribute to gum disease.
4. The dental professional may also:
5. Take an x-ray to see whether there is any bone loss.
6. Refer you to a periodontist. "Periodontists are experts in the diagnosis and treatment of gum disease and may provide you with treatment options that are not offered by your dentist".[20,21,22,23,24,25,26]

Treatment

Periodontitis can be treated by various ways, based on its stiffness. periodontitis management is to completely clean the bacterial pockets to prevent more harm. Periodontitis supervision can be carried out by a periodontist, dentist and dental hygienist with strict daily routine of good oral care.

Treatment for Periodontitis can be done by two methods

1. Mechanical Therapy

Elimination or adequate suppression of periodontal pathogens in subgingival microflora is essential for healing to take place. In general periodontal disease can be treated by Mechanical therapy successfully by scaling and root planning and the results of therapy can be maintained over prolonged periods of time by regular meticulous oral hygiene.

2. Systemic Antibiotic Therapy

An ideal antibiotic for use in prevention and treatment of periodontal diseases should be specific for periodontal pathogens, allogenic and nontoxic, substantive and inexpensive. Combination of antibiotics may be necessary to eliminate all putative pathogens from some periodontal pockets.

On The Basis Of Surgical/Non Surgical Treatment

(I). Surgical treatment

Gingivectomy

A gingivectomy removes and reshapes loose, diseased gum tissue to get rid of pockets between the teeth and gums. A gum specialist (periodontist) or oral surgeon often will do the procedure. The doctor will start by numbing your gums with a local anesthetic. He or she may use a laser to remove loose gum tissue.

Gingivoplasty

Although gingivectomy was initially developed to treat periodontal disease, it is now a common Cosmetic Surgery, also. It is used to remove overgrown gum tissue and improve the appearance of the gums. Gingivoplasty is the surgical reshaping of gum tissue around the teeth.

1. Flap surgery/pocket reduction surgery: For advanced stages of periodontal disease, a surgery may be required. During this surgical procedure, the gums are lifted back and tartar is removed. In some situations, irregular surfaces of damaged bone are smoothed where disease-causing bacteria may be hiding. The gums are then placed so that the tissue will fit snugly around the tooth. This reduces the space between the gum and tooth, which reduces the chance of bacteria from growing.
2. Bone grafts: This procedure uses fragments of your own bone, donated bone, or synthetic bone to replace bone destroyed by gum disease. This promotes regrowth of bone, which makes the teeth more stable. Soft tissue grafts can also be used to reinforce thin gums or to fill in places where the gums may have receded.
3. LANAP laser gum surgery: This surgery is used by our periodontal specialists because it is less painful, reduces sensitivity post-op, and requires very little downtime after treatment compared to traditional osseous surgery.

(II). Non-surgical treatment

1. Dental cleaning: During the routine dental cleaning, plaque and tartar are removed from above and below the gum line of the teeth. If any signs of gum disease are suspected, we may recommend more frequent dental cleanings.
2. Scaling and root planing: For cases that are found early, scaling and root planing is used. This deep-cleaning and nonsurgical procedure is done under local anesthesia. Plaque and tartar are scraped away from above and below the gumline, and rough spots on the tooth root are smoothed with planing. Smoothing these rough spots removes bacteria and provides a clean surface for the gum to reattach properly to the teeth.[27,28,29,30]

Formulation

Table 1.2: Formula of clove oil gel.

Ingredients	F1	F2	F3	F4	F5
Clove oil (ml)	0.75	0.75	0.75	0.75	0.75
Carbopol (g)	0.3	0.4	0.5	0.6	1
Poly ethylene glycol (ml)	15	15	15	15	15
Glycerin (ml)	5	5	5	5	5
Methyl paraben (g)	0.18	0.18	0.18	0.18	0.18
Propyl paraben (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	q.s	q.s	q.s	q.s	q.s

Procedure

Formulation of clove oil gel

Carbopol 934 gels were prepared by soaking carbopol 934 in water and by neutralizing with triethanolamine to pH 6.4. Weighed amount of methyl and propyl paraben were added to the water before the addition of carbopol 934.[34]

In another beaker, the required quantity of propylene glycol was taken in another test tube to which accurately measured the amount of clove oil corresponding to its MIC was incorporated and finally the mixture was added to the beaker containing carbopol with stirring.[35] The sweetening agent was also added to the polymer dispersion and stirred continuously till it forms a homogenous product.[36] The volume was made up with distilled water and stirring was done vigorously. All the gels were then subjected to evaluation tests in order to select the best formulation.

Formulation Of Clove Oil Gel

Table 1.3: Process of formulation of clove oil.

1.	**Soaking**	Soak carbopol 934 in water
2.	**Neutralization**	Neutralize with triethanolamine to pH 6.4
3.	**Addition of preservatives**	Addition of methyl paraben and propyl paraben
4.	**Addition of co solvent and API**	Addition of propylene glycol and clove oil in a other test tube
5.	**Addition of sweetener**	Finally aspartame is added
6.	**Stirring**	Stirring is done until homogeneous product is formed

Introduction to ingredients

 # Carbopol 934 polymer

Carbopol 934 polymer is a white powder, cross-linked polyacrylic acid polymer. It exhibits short flow properties and a creamy sensory profile, and is therefore well suited for use as a rheology modifier in lotions and creams.

 # Polyethylene glycol

Polyethylene glycol (PEG) is a polyether compound with many applications, from industrial manufacturing to medicine. PEG is also known as polyethylene oxide (PEO) or polyoxyethylene (POE), depending on its molecular weight. The structure of PEG is commonly expressed as $H{-}(O{-}CH_2{-}CH_2)_n{-}OH$.

 # Glycerin

Glycerin is a trihydroxyalcohol with localized osmotic diuretic and laxative effects. Glycerin elevates the blood plasma osmolality thereby extracting water from tissues into interstitial fluid and plasma. This agent also prevents water reabsorption in the proximal tubule in the kidney leading to an increase in water and sodium excretion and a reduction in blood volume.

 # Methyl paraben

Methylparaben is a 4-hydroxybenzoate ester resulting from the formal condensation of the carboxy group of 4-hydroxybenzoic acid with methanol. It is the most frequently used antimicrobial preservative in cosmetics. It occurs naturally in several fruits, particularly in blueberries. It has a role as a plant metabolite, an antimicrobial food preservative, a neuroprotective agent and an antifungal agent.

 # Propyl paraben

Propylparaben is the benzoate ester that is the propyl ester of 4-hydroxybenzoic acid. Preservative typically found in many water-based cosmetics, such as creams, lotions, shampoos and bath products. Also used as a food additive. It has a role as an antifungal agent and an antimicrobial agent. It is a benzoate ester, a member of phenols and a paraben. It derives from a propan-1-ol and a 4-hydroxybenzoic acid.

 # Aspartame

Aspartame is a dipeptide obtained by formal condensation of the alpha-carboxy group of L-aspartic acid with the amino group of methyl L-phenylalaninate. Commonly used as an artificial sweetener. It has a role as a sweetening agent, a nutraceutical, a micronutrient, a

xenobiotic, an environmental contaminant, an apoptosis inhibitor and an EC 3.1.3.1 (alkaline phosphatase) inhibitor. It is a dipeptide, a carboxylic acid and a methyl ester. It derives from a L-aspartic acid and a methyl L-phenylalaninate.

 # Distilled water

Distilled water is water that has been boiled into vapor and condensed back into liquid in a separate container. Impurities in the original water that do not boil below or near the boiling point of water remain in the original container. Thus, distilled water is one type of purified water.

Role of Clove and Clove Oil

Common Names:- Cloves, Carophyllus, Clovos, Caryophyllus

Botanical Names:- *Eugenia caryophyllus, Syzygium aromaticum*

Table 1.4: Taxonomical classification of clove.

Domain	**Eukaryota**	**Subclass**	**Rosidae**
Kingdom	Plantae	Super order	Myrtanae
Subkingdom	Viridaeplantae	Order	Myrtales
Phylum	Tracheophyta	Suborder	Myrtineae
Subphylum	Euphyllophytina	Family	Myrtaceae
Infraphylum	Radiatopses	Genus	Syzygium
Class	Magnoliopsida	Specific epithet	Aromaticum

Clove is known to possess antibacterial properties and is been used in various dental creams, tooth pastes, mouth washes, and throat sprays to cleanse bacteria. It is also used to relive pain from sore gums and improves overall dental health. In dentistry, eugenol in combination with zinc oxide is used for temporary filling of cavities. Clove is an anodyne (an agent that soothes or relives pain) for dental emergencies.

Clove is used as an anti-inflammatory agent, due to its high content of flavonoids.Paste of clove powder in water promotes faster healing of cuts and bites.

Clove and clove oil boosts the immune system by purifying the blood and helps to fight against various diseases.

Clove may be looked upon as the champion of all the anti-oxidants known till date. The Oxygen Radical Absorption Capacity test (ORAC) is a scale developed by U.S. Department of Agriculture for comparing anti-oxidant activity. The ORAC score, of clove is over 10 million. A drop of clove oil is 400 times more powerful as an anti-oxidant than wolf berries or blueberri.

Preparation of Gelling Agent (Carbopol)

Appropriate quantity of carbopol 934 was soaked in water for a period of 2 hours. Carbopol was then neutralized with triethanolamine (TEA) with stirring. Then specified amount of drug was dissolved in appropriate and pre -weighted amounts of propylene glycol and ethanol. Solvent blend was transferred to carbopol container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min, finally adjusted the pH with 98% TEA until the desired pH value was approximately reached (6.8-7). During pH adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hours at room temperature prior to performing rheological measurements.

Other gel formulations were prepared by dispersing 3% w/w HPMC, 5% w/w Na CMC and 8% w/w sodium alginate in water by continuous stirring for a period of 2 h. Aceclofenac was dissolved in propylene glycol or ethanol or isopropyl alcohol and the solution was added gently to HPMC, Na CMC and sodium alginate dispersion under continuous stirring. The mixture was stirred gently with a spatula until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 h at room temperature prior to performing rheological measurements.[4]

Preparation of Gel

Carbopol 934 gels were prepared by soaking carbopol 934 in water and by neutralizing with triethanolamine to pH 6.4. Weighed amount of methyl and propyl paraben were added to the water prior to the addition of carbopol 934. In another beaker, the required quantity of propylene glycol was taken in another test tube to which accurately measured the amount of clove oil corresponding to its MIC was incorporated and finally this mixture was added to the beaker containing carbopol with stirring. The sweetening agent was also added to the polymer dispersion and stirred continuously till it forms a homogenous product. The volume was made up with distilled water and stirring was done vigorously. All the prepared gels were then subjected to evaluation tests in order to select the best formulation. The composition of different gel formulations is listed in Table.[14,15]

Pharmacological Properties of Eugenol

Eugenol (C10H12O2 or CH3C6H3) is a volatile phenolic constituent of clove essential oil obtained from *Eugenia caryophyllata* buds and leaves.

The name supposedly is derived from the scientific name for clove *E. caryophyllata* tree which has large leaves and flower buds which turn to red color when they are ready for collection. Eugenol is the main extracted constituent (70-90%) of cloves and is responsible for clove aroma.

Eugenol, a phenylpropanoid, is pale yellow oil with a spicy aroma with the molecular weight of 164.2 g/mol. This molecule is a weak acid which is soluble in organic solvents and specially extracted from clove oil, nutmeg, cinnamon, basil and bay leaf.

Antibacterial activities of eugenol

The effects of eugenol on the growth of some species of Gram-positive (*Bacillus cereus; Bacillus subtilis; Staphylococcus aureus) and Gram-negative (Escherichia coli; Salmonella typhi; Pseudomonas aeruginosa)* bacteria were assessed by using the agar well diffusion method. Eugenol has shown an inhibitory effect on the growth of the P. aeruginosa at the concentration of 1000 µg/mL. The complete inhibitory effect against such bacteria is shown at 2000 µg/mL. In this study, ampicillin (1 mg/mL) is used as positive control and similar effects of eugenol also have confirmed against various pathogens such as E. coli, B. cereus, Helicobacter pylori, S. aureus, Staphylococcus epidermidis, Streptococcus pneumoniae and Streptococcus pyogenes.

The combination of eugenol with a conventional antibiotic has been evaluated to detect the synergistic effect against Gram-negative bacteria. In the eugenol treated cells, 50% loss of membrane integrity was demonstrated which enhanced the activity of studied antibiotics. The combination of eugenol with two antibiotics, vancomycin and a β-lactam, showed an increased membrane damage in bacteria which means a synergistic effect. It has also been demonstrated that penetration of vancomycin and β-lactam, in combination with eugenol, has increased and resulted in more antimicrobial effect.

Anti-inflammatory effects of eugenol

Investigations of anti-inflammatory effects of eugenol, have suggested that this compound is able to suppress the expressions of cyclooxygenase II enzyme. Eugenol dimers can inhibit the expression of cytokines in macrophages, which are stimulated by polysaccharides. Eugenol also has an inhibitory effect on cell proliferation via suppression of NF-Kappa B (NF-kB). Eugenol suppresses the activation of NF-kB which induces reduction in the incidence of gastric tumors. Eugenol can also stimulate the expression of NF-kB target genes which are responsible for the regulation of cell proliferation and cell survival. Because of these suggested activities, eugenol has been indicated to have chemo preventive effect.[3]

Physiological properties of eugenol oil

Table 1.5: Physiological classification of clove oil.

Sr. No.	Parameter Eugenol oil (Ref)	Eugenol Oil (Std)
1 Colour	Pale Yellow	Pale Yellow
2 Odor Aromatic	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	1.02g/ml	1.06g/ml
7 Refractive Index	1.492	1.532

Toxicological Properties of Eugenol

Eugenol is considered safe as a food additive, but due to the wide range of different applications and also the extensive use and availability of clove oil; there is a great concern about its toxicity in recent years.

Cytotoxicity of eugenol

The cytotoxic effects of eugenol, induction of reactive oxygen species (ROS) production and reduced levels of GSH(Glutathione) have been studied in human submandibular cell line. It is suggested that formation of benzyl radicals is the main cause of low GSH of eugenol is found to be related to ROS-independent mechanisms. Eugenol has been found to exert less cytotoxic effects compared to isoeugenol and such effects are dose dependent.

Eugenol was also found potential to decrease the activity of dehydrogenase enzymes in human osteoblastic cells in a dose dependent manner. The cytotoxic effects of some of root canal sealer agents based on zinc-oxide eugenol (endofill) and sealer 26 were also studied. Results showed that both agents have cytotoxic effects, but the toxicity of "sealer 26" on macrophages is more than endofill. Anpo et al. evaluated the cytotoxic effects of eugenol on human pulp cells and also the expression of molecular markers in osteogenic differentiation. Observations suggested that eugenol used for endodontic treatment, may have cytotoxic effects on the normal function of stem cells.

Genotoxicity of eugenol

Authors have suggested that a moderate to severe toxic effects of zinc oxide eugenol in V79 cell line and also demonstrated that these effects are dose dependent,

suggesting that eugenol has genotoxic effects.[41] On the other hand, the chemopreventive effect of eugenol on DNA damage induced by 7,12 dimethylbenzanthracene (DMBA) has been evaluated in MCF-7 cells. The observations suggested that eugenol was potent to protect DNA against genotoxic damage induced by DMBA. Eugenol is able to suppress the DMBA activation and acts as a potential chemopreventive compound.

Immunotoxicity of eugenol

Findings about the potency of eugenol and clove oil in inducing allergy and hypersensitivity are controversial. Several adverse effects have been observed that after use of dental products which contain eugenol. Localized irritation of the skin; ulcers, allergic dermatitis, tissue necrosis and rarely even anaphylactic-like shock have been reported in different studies.

The allergic capacity of eugenol containing fragrance was evaluated in approximately 24.000 individuals. Findings reported that 25.5% of health care workers, 16.5% of non-health care workers, 39.39% of metal workers and 16.3% of people in other occupations showed allergic reactions to eugenol.[3]

Evaluation

Physical appearance

- Color:

The color of the formulation developed was checked out against a white background.

- Consistency:

The consistency was checked by applying formulation on the skin.

- Greasiness:

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

- Odour:

The odour of the gels was checked by mixing the gel in water and taking the smell.

pH determination

Digital pH meter was used to determine the pH of the formulations by dipping the electrode in 25 ml of distilled and deionized water containing 2g of formulated clove oil loaded dental gel. The pH measurements were performed three times, and the average was noted as the result depicted for each formulation.[5,6]

Viscosity determination

Viscosity determination was carried out using Brooke-field viscometer (TV-10) which was connected with a thermally-controlled water bath that maintained the temperature at 37°C. Prior to each experiment, the viscometer was equilibrated with the sample for 10 min and at a shear rate of 20RPM. The viscosity measurements obtained was taken as the average of three readings for each formulation (n = 3).[10]

Gelation temperature measurement

Gelation temperature was determined by following tube tilting method.[8] In this method 2g of aliquots of formulated gel was transferred to a test tube covered with the aluminium foil and was immersed in a temperature controlled water bath at 40°C. The samples were heated by increasing the temperature of water bath by 1oC till there was stable meniscus on 90otilting of the test tubes after equilibration for 5 min at every 10°C temperature increased. The data obtained was taken as the average of three readings for each formulation (n = 3).[10]

Determination of extrudability

It was determined by using a tube filled with the gel, having a tip of 5mm opening and by measuring the amount of gel that extruded through the tip when a pressure was applied on the tube and readings were noted down.

Determination of homogeneity

All the prepared gels were tested for homogeneity by visual inspection after the gels have been set in the container.[11,13] They were tested for their appearance and presence of any aggregates.

Determination of drug content

The drug content of the gel formulations was determined by dissolving an accurately weighed quantity 1 g of gel in 100 ml of solvent (a mixture of ethanol and phosphate buffer pH 6.8 for the formulation of clove oil). The solutions were kept for shaking for 4 hr and then kept for 6 hr for complete dissolution of the formulations. Then the solutions were filtered through 0.45 mm membrane filters and proper dilutions were made and solutions were subjected to the spectro photometric analysis. The drug content was calculated from the linear regression equation obtained from the calibration data.[11,12]

Swelling index studies

Swelling index studies were performed at 37°C using a thermo jacketed cell containing phosphate buffer saline pH 6.8 as swelling medium. About 2g of clove oil loaded dental gel was taken and the study was carried out for 6 h. After 6hour, the gels were scrapped /removed from aluminium foil and the weight was determined by removing the adhered water from the surface by blotting immediately. The swelling Index of the dental gel forming polymer was determined using the relationship[7]

$$\%St = (Wt - Wo)X100 \div Wo$$

Where 'St' represents the swelling of the dental gel at time't'.

W0 and Wt represent the initial and final weight of gelling solution and gel respectively.

All measurements were performed in triplicate (n=3) and the data noted is the average of the three readings.

Determination of spreadability

Spreadability study was performed by determining the "Slip" and "Drag" characteristics of clove oil loaded

dental gel using a wooden block consisting of a pulley at one end. The formulations were kept on one slide and sandwiched by another slide across a length of 6 cm among the two slides. A uniform thin layer was formed by placing a 100 gm weight upon the upper slide. Then the weight was removed, and the adhered excess formulation to the slide was scraped off and a 20 gm loaded pulley was used to slip the upper slide to 6 cm distance and to separate away to the direction of applied force from the slide fixed on the board of the apparatus at the bottom. The time taken for complete separation of the two slides was recorded. The experiment was repeated for three times and the average was noted for each formulation. A shorter interval indicates better spreadability [9].

Spreadability = m x l/t

Where, 'm' represents the weight tied to the upper slide (20 gm)

'l' represents the length of glass slide (6 cm)

't' represents the time taken to separate the upper slide from the lower slide.

Stability study

The stability study was a determination as per ICH guidelines. The formulated gel was filled in collapsible tubes and hold on at completely different temperatures and humidness conditions, viz. 25ºC± 2ºC /hour five-hitter RH, 30º C ± 2ºC / 65% ± 5% RH, 40ºC ± 2ºC / 75% ± 5% RH for three months and studied for appearance, pH and spreadability.

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.[31] Then the solutions were filtered through 0.45 mm membrane filters and proper dilutions were made and solutions were subjected to the spectrophotometric analysis.[32] 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 clove oil gel. All formulations of clove oil gel of about 2% were placed aseptically in cups of agar plate which was previously inoculated with culture. The plates were left at ambient temperature for 30 mins prior to incubation at 37◦C for 24 hrs. The broad spectrum antibiotic i.e., tetracycline was used as positive control for obtaining comparative results. Plates were observed after 24-48 hrs incubation for the appearance of the zone of iinhibitio. Antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (millimeters) of microbial growth.[33]

CONCLUSION

The clove oil was found to have antimicrobial activity against *Streptococcus salivarius, Streptococcus sanguis, Lactobacilli acidophilus*. 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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