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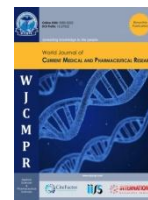
A herbo health care for Gums and Throat

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Abstract

The use of herbal gum paint in the treatment of dental problems is not very common. Most of the drugs used are synthetic and are in used over the years mainly for antibacterial activity in the oral cavity. It includes various side effects that are always a limit for their use. Natural remedies are always more desirable and acceptable and are more effective with fewer side effects. The microorganism includes Escherichia coli, Klebsiella pneumonia, Staphylococcus epidermidis, and staphylococcus aureus which cause gingivitis and dental cavities. The current study objective is to formulate and evaluate an herbal gum paint that is useful in the long term and has rheological characteristics and stability too. The local anesthetic activity is studied and the antimicrobial activity of gum paint is examined by disc diffusion method and compared activity between F1-betel leaf oil, F2- clove oil, and an F3 (F1+F2) – both betel leaf and clove oil. F3 shows a synergistic effect on microbes than the other two and has the maximum zone of inhibition.

Introduction

From ancient times to recent times, dental infection is the most common problem worldwide. The dental infection doesn't depend on the age group. The major factors that contribute to dental infections are lifestyle changes, lack of intake of adequate nutrition, improper oral hygiene, etc. Our ancestors mostly treated oral problems with self-made home remedies. The most commonly available and most promising remedies used are clove and betel leaf as they formed a part of our daily food. The whole part shows many major medicinal uses1. It is popular in ayurvedic practice; clove is inserted into an infected cavity or else applied as a topical extract to relieve pain and inflammation. It also exerts potent anti-bacterial properties that can aid in healing and prevent infection. Clove oil is found equally effective against both gram-positive and gram-negative organisms. Thus from this study, it concludes that clove oil possesses major anti-bacterial action against clinical isolates of *E.coli*. Gum tooth paint has been used as anodyne, an anti-inflammatory for toothache, and mainly after tooth extraction. Oral care is an important key to maintaining the health of teeth. The tooth consists of the crown, root, enamel, dentine, and pulp. Toothache occurs when the innermost layer of the tooth (dental pulp) becomes inflamed. The pulp is made up of sensitive nerves and blood vessels. Dental pulp can inflame due to microbial infection, which mostly occur because of cavity. Tooth paint and gel act as an ideal vehicle for any active ingredient. Among these, gum paint serves as the most accessible, promising anodyne and anti-inflammatory action on oral care. Due to certain side effects of routinely non-herbal gum paint, people restore to herbal paint, which doesn't

contain any synthetic ingredients. Consumers who gravitate to using herbal products often view their products as safer than product that contains chemicals. Their efficacy can be attributed to various actions. The use of products to improve oral health dates back to ancient and continues up to nowadays, with hundreds of marketed products ruling the medical products. Those products may be both synthetic [2,3,4] and a natural [5,6,7] – the ingredients are thymol, tannic acid, menthol, iodine, potassium iodide, glycerin, zinc chloride, and so on and the natural are neem, clove, turmeric, camphor, cinnamon, catechu and so on. Betel leaf8has been known by the people of Indonesia for a long time, as an ingredient that would strengthen teeth, stop gingival bleeding, and as a mouthwash remedy. Betel leaf can stop the bleeding, skin wound healing, gastrointestinal drugs (stomachic), and clear the throat. Betel leaf also has the properties of anti-septic, anti-oxidant, and anti-fungal activity. Essential oil and its extract can fight against gram-positive and negative bacteria. Phenol and chavicol are the major active substances in betel leaf essential oil.

Dental problems are considered a major diverse part of dental health. Dental pain is the most intolerable one when occur mostly after tooth extraction. Tooth extraction involves both simple and surgical extraction. Before it, a local anesthetic is administered, which helps to loosen the tooth in the gums. It may feel the pressure without pain. The local anesthetic activity long as long as the extraction procedure. After some time, it disappears which make the people feel a pain that is vulnerable to its control even though prescribe over -the-counter drugs such as gel form and so on. Gum paint is also one of the best formulations. Medicated gum paint is applied as a

topical solution as locally on gums for several bacterial infections and inflammatory conditions. It also acts as effective healing property and easily get absorbed in gums. Commercially available gum paint has side effects such as burning sensation, stinging sensation, nausea, etc. After tooth extraction, many bacteria like *Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis,* and *Staphylococcus aureus* produce infection where the open socket is more prone to it.

A study has demonstrated the use of herbal clove oil and betel leaf oil as an effective local anesthetic, anodyne, anti-inflammatory and anti-microbial activity formulated as an herbal gum paint with reduced side effects. A study also focuses on the exposure of lower levels of betel leaf oil and clove oil and both combined form which demonstrated anti-microbial activity on above - mentioned microbes.

Materials and Methods

Clove oil gift sample collected from Raga Naturals Pvt ltd., Virudhunagar district, Tamil Nadu.

Betel leaf oil purchased from Amazon.

HERBAL INGREDIENTS PROFILE [9,10]

S.no	Ingredients	Botanical Name	Parts Used	Category
1.	Clove Oil	*Syzygium aromaticum*	Flower bud	Dental uses as a local anesthetic, Anti-bacterial, Anti-inflammatory
2.	Betel Leaf Oil	*Piper betle*	Leaves	Anodyne, Anti-bacterial, Analgesic, Anti-inflammatory

1. Evaluation

Organoleptic characteristics of Clove oil

Color   : Colorless or pale yellow.

Odor   : Strong aromatic odor of clove

Taste   : Pungent spicy taste.

Chemical test of clove [11]

Treatment of clove oil with potassium hydroxide solution (50%) formed needle-shaped crystals of potassium eugenate.

To clove oil (1ml) in alcohol (5ml) added ferric chloride solution (1ml). The solution turned blue due to the presence of the phenol hydroxyl group.

Added ferric chloride solution to the aqueous extract of the clove. The solution turned into Blue-black due to the presence of tannins.

Organoleptic characteristics of betel leaf oil [12]

Color   : Golden Yellow

Odor   : Aromatic

Taste   : Pungent Solubility: Toluene, Ethanol.

Chemical test of Betel leaf [13]

About 50mg of sample dissolved in 5ml of distilled water. To this neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

Photochemical Analysis [14]

Photochemical components of essential oils in clove leaf and betel leaf were screened by using standard methods. The components analyzed showing presence of eugenol in both betel leaf and clove oil by Thin Layer Chromatography and Phenol in betel leaf oil by UV-Vis spectroscopy.

Analysis of Eugenol by Thin-layer chromatography in Clove and Betel leaf oil Eugenol is a member of allyl benzene class of chemical compounds. It is present in various essential oils like clove, nutmeg, betel leaf and so on. The most common and feasible analytical tool is Thin Layer Chromatography.

Each essential oil (clove oil and betel leaf oil) was diluted in chloroform/toluene as a ratio (1:10). Eugenol was applied as a standard and it is diluted at a 1:30 ratio and 10μl of each should be applied as a spot on the TLC plate. It was placed in a development chamber of toluene: ethyl acetate and run a TLC plate for 10cm. After it, sprayed thoroughly with vanillin-sulfuric acid and heat a plate at 110∘ C for 5-10 min under observation. The eugenol appears as orange - brown and the Rƒ value was calculated.

Ultraviolet-Visible spectrum analysis of phenolic content in Betel leaf oil [15]

The phenolic content in betel leaf oil was analyzed against a standard - gallic acid. Gallic acid is a poly phenol produced by plants and commonly used to measure polyphenol concentration and gives better responses amongst all phenolics tested.

Standard preparation

The standard was prepared by using 0.01mg gallic acid and dissolved it in water and made up to 10ml. From it, took 1ml Gallic acid in test tube and added 0.5ml folin- ciocalteau reagent (prepared by diluting 1ml reagent in 9ml water) and waited for 15 minutes. After it, added 1ml of sodium carbonate to it and kept aside for 30 minutes, and made dilutions as 1ml to 10ml of the above sample with water and made up to volume 10ml. The absorbance of all samples was measured at 765nm using a UV - Visible spectrophotometer.

Sample preparation

Pipetted out 0.11ml of betel leaf oil (considering specific gravity for 1ml = 0.8978) and dissolved in ethanol and made up to 100ml. Took 1ml from the above solution and added 0.5ml of reagent (prepared by diluting 1ml reagent in 9ml water) and waited for 15 minutes. After it, added 1ml of sodium carbonate to it and kept aside for 30 minutes, and made dilutions as 1ml to 10ml of the above sample per 10ml of water. The absorbance of all samples was measured at 765nm using a UV-Visible spectrophotometer.

Formulation of Gum Paint

Each 2mL of essential oil (Betel leaf and Clove oil) was dissolved in 2mL ethanol in the beaker and considered the first component. Span 80 - 3mL was added to the first component in a dropwise manner and stirred until it was miscible. 6mL of glycerin was separated into 2 parts each of 3mL which was taken in two separate beakers. Added this first component as drop by drop in 2 parts of each 3ml of glycerine in two separate beakers and stirred for each drop and made a formulation as

much as viscous. Then, combine those two parts of 6mL glycerin in two beakers to one part and stirred it for almost 5 minutes and a light whitish appearanceformulation was obtained.

Formulation development

The formulation of the lab-made polyherbal gum paint was achieved by studying various herbalformulation articles. Totally 3 trials were made with modifications in the formula to give the best formula.

The various trials made are elaborated on in the following sections.

S. No	Ingredients	F1	F2	F3
1	Clove oil	---	3ml	2ml
2	Betel leaf oil	3ml	---	2ml
3	Span 80	3ml	3ml	3ml
4	Glycerin	7ml	7ml	6ml
5	Ethanol	2ml	2ml	2ml

Formulation 1 (F1-Betel leaf oil)

In poly herbal gum paint, the selected ingredients were glycerin and ethanol assolvent and span 80 as surfactant. Betel leaf oil was dissolved in ethanol to form a miscible mixture and glycerin was separated into 2 parts. Span 80 dissolved in the above mixture drop by drop to form a miscible compound. This miscible compound was dissolved in two respective glycerine parts drop by drop and combined to make a final compound. The final product appeared as a light whitish.

Formulation 2 (F2-Clove oil)

In poly herbal gum paint, the selected ingredients were glycerin and ethanol assolvent and span 80 as surfactant.

Clove oil dissolved in ethanol to form a miscible mixture and glycerin was separated into 2 parts. Span 80 dissolved in the above mixture drop by drop to form a misciblecompound. This miscible compound was dissolved in two respective glycerin parts drop by drop and combined to make a final compound. The final product appeared as a light whitish.

Formulation 3 (F3- Betel leaf oil and Clove oil)

In poly herbal gum paint, the selected ingredients were glycerine and ethanol assolvent and span 80 as surfactant.

Betel leaf oil and clove oil were dissolved in ethanol to form a miscible mixtureand glycerine was separated into 2 parts. Span 80 dissolved in the above mixture drop by drop to form a miscible compound. This miscible compound was dissolved in two respective glycerin parts drop by drop and combined to make a final compound. The final product appeared as a light whitish.

Physiochemical Characteristics of Formulated Gumpaint [16]

The prepared formulations were subjected to the following parameters:

Appearance

Determination of pH

Spreadability

Viscosity

ANTI-MICROBIAL ACTIVITY OF EUGENOL CONTENT IN BOTH BETEL LEAF AND CLOVE OIL AGAINST HUMAN PATHOGENS [17]

Anti-microbial activity of piper betel leaf oil and clove oil against different human pathogens of both gram-positive and gram-negative bacteria. The significant activity was observed and recorded. The known low-level concentration (10µl) of the formulation was inoculated in the respective medium. The anti-microbial activity against respective organisms such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, and Staphylococcus aureus* was obtained from microbial type culture. Disc method was used to investigate the anti-microbial activity. The pure culture of bacteria was grown in a respective medium such as MacConkey agar (*Escherichia coli*), Muller-Hinton agar (Klebsiella *pneumonia*), and Mannitol salt agar (*Staphylococcus epidermidis and Staphylococcus aureus*).Amikacin was used as a standard or control.

Results and Discussion

In the present study, an attempt had been made to prepare medicated gum paint using herbal ingredients. Our formulation was the drug of choice showing promising anodyne effect,anti-bacterial and anti-inflammatory effects in people especially who had undergone tooth extraction process. A total of three medicated gum paint preparations were prepared and evaluated for various parameters, i.e.

Appearance

Light whitish

Thin – layer chromatography

Eugenol content in clove and betel leaf oil were estimated as follows,

Compounds	Distance Travelled by the compound	Distance travelled bythe solvent front	Rf value = $\dfrac{\text{Distance travelled by the compound}}{\text{Distance travelled by thesolvent front}}$
Standard-Eugenol	0.68	0.90	0.7
Samples – Clove oil	0.65	0.9	0.71
Betel leaf oil	0.66	0.93	0.71

Ultraviolet-visible spectroscopyStandard

concentration(µl/1ml)	Absorbance
1	0.082
2	0.124
3	0.175
4	0.238
5	0.264

Sample

concentration(µl/1ml)	Absorbance
1	0.042
2	0.091
3	0.117
4	0.200
5	0.230

STANDARD CURVE OF GALLIC ACID

$y = 0.0053x + 0.0148$
$R^2 = 0.985$
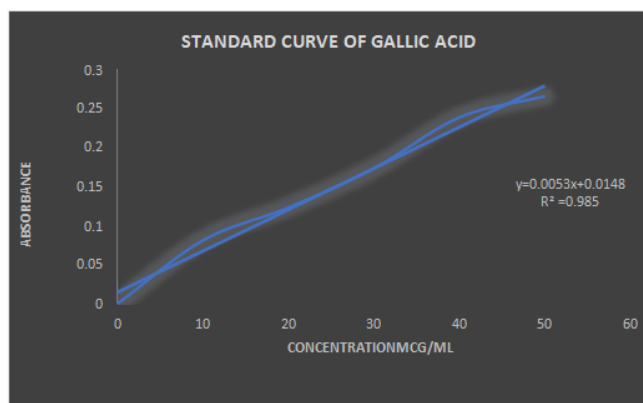
Total phenol content in betel leaf oil as follows,

S.no	Concentration Gallic acid & betel leaf oil(µg/ml)	Absorbance	
		Gallic acid *mean±sem	Betel leaf oil *mean±sem
1	10	0.081±0.046	0.041±0.023
2	20	0.123±0.071	0.092±0.053
3	30	0.173±0.099	0.118±0.068
4	40	0.238±0.137	0.201±0.116
5	50	0.265±0.153	0.231±0.133
		GAE	81.58mg/gm

Physical evaluation of formulation comparison report

Formulations	pH	Viscosity(cps)	Spreadability (g.cm/s)
F1	6.5	11750	11.20± 0.6
F2	6.7	11990	11.26±0.65
F3	6.8	12220	11.35±0.61

TABLE: 1

Determination of pH

The pH of the prepared gum paint was found to be 6.5 to 7.0 as shown in table 1.

Viscosity

The mean average viscosity of prepared gum paint was found to be 11750to12220 cps as shown in table 1.

Spreadability

The spreadability of prepared gum paint was found to be 11.20±0.6 to11.35±0.61 g-cm/sec as shown in table 1.

Anti-microbial studies:

Tabulation showed the **zone of inhibition** diameter against standard Standard Disc: Amikacin – 18mm zone of inhibition

| Human Pathogens	F1(mm)	F2 (mm)	F3(mm)
Escherichia coli	23	20	24
Klebsiella pneumonia	30	20	35
Staphylococcus epidermidis	20	25	30
Staphylococcus aureus	-	-	40

Table: 2

From the studies, it showed that the formulation F3 had highly maximum inhibition than the other two F1 and F2 Escherichia Coli,

F3 had a **24mm** zone of inhibition diameter as shown in table 2.

Klebsiella pneumoniae,

F3had a **35 mm** zone of inhibition diameter as shown in table 2.

Staphylococcus epidermidis,

F3 had a **30mm** zone of inhibition diameter as shown in table 2.

Staphylococcus aureus,

F3 had a **40mm** zone of inhibition diameter as shown in table 2.

The F3 – betel leaf oil and clove oil combination showed the maximum zone of inhibition among all the two F1 and F2. It showed that F3 had synergistic activity against the human pathogens i.e., ***Escherichia Coli, Klebsiella pneumonia, Staphylococcus epidermidis,*** and ***Staphylococcus aureus.***

The pictorial representation of anti-microbial activity of F3 be

Escherichia coli	Klebsiella pneumonia	Staphylococcus epidermidis	Staphylococcus aureus
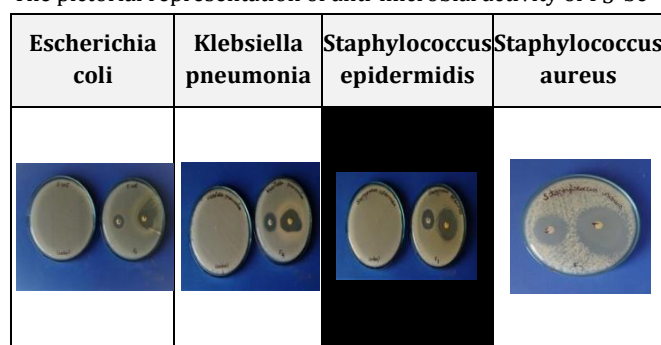
Stability studies

There was no such change in stability.

Conclusion

In the past decade, homemade remedies and materials in the kitchen gain more value and the value-added products reach society because of their availability, ease of processing, and being devoid of unwanted effects these technological advancements in bringing information to society. In this project, we have focused on herbal gum paint containing a strong spicy aroma, andactive ingredients. Clove oil and betel leaf oil with the synergistic activity of anodyne, anti-inflammatory and antibacterial activity. Poly herbal gum paint was formulated using clove oil and betel leaf oil as active ingredients. Essential oils present in gum paint had been purchased commercially and were standardized by chemical investigation. Its drives consist of the active ingredients and span 80 as the stabilizing agent.

Poly herbal gum paint shows pH which is non-irritable with excellent viscosity and spreadability. The focus of gum paint on antimicrobial activity against, *Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, and Staphylococcus aureus* exhibits a wide range of activity. A safe, herbal formulation with a standardized protocol for storage and expected usage of antimicrobial activity was formulated and standardized.

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