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PREPARATION OF ANTIMICROBIAL CHEWING GUM TO TREAT HALITOSIS

Bhumesh P. Lilhare*, Dr. Manjeet Singh, Dr. Rajesh Z. Mujariya, Lokesh I. Patle.

Student, Executive Director, Principal, HOD Pharmaceutics.
Department of Pharmaceutics,
Sardar Patel University, Balaghat

Abstract: The aim of the current study was to evaluate the antimicrobial activity of the *Eugenia Caryophyllus* oil and *Trigonella foenum-graceum L.* The aqueous flower bud extracts of *Eugenia Caryophyllus* and aqueous seed extract of *Trigonella foenum-graceum* prepared and tested qualitatively, by screening of phytoconstituents that present in the extracts and to quantitatively by checking antimicrobial activity using well diffusion agar plate method. Agar cup plate test was used to determine the sensitivity of the tested samples while the well microdilution was used to determine the minimum inhibitory concentration. The drug extract were extracted using solvent extraction by soxhlet apparatus, different concentration of these extract were prepared ranging from 0.1-1.0 ml and antimicrobial activity is performed on *Staphylococcus aureus* (gram positive) bacteria, using nutrient agar as broth. It was found that the above drug combination *Eugenia Caryophyllus* & *Trigonella foenum-graceum* on the respective gram positive bacteria *Staphylococcus aureus* was showing optimum antimicrobial activity.The minimum inhibitory concentration of combination of both drugs was found to be 8.0 mm for the concentration of 0.9 ml and 1.0 ml for both drugs Clove oil & Fenugreek seed Extract.

Chewing gum preparation shows antimicrobial activity in the range of **4.8mm to 5.5mm.**
The overall results of this study indicate that the chewing gum prepared from both the drug extracts have optimum antimicrobial and potential for treating Halitosis.

Key Words: **Chewing gum, Eugenia Caryophyllus, Trigonella foenum graceum, MIC: Minimum Inhibitory Concentration, Halitosis, Staphylococcus aureus.**

I. INTRODUCTION:

Halitosis: - Human breath is composed of highly complex substances with numerous variable odors which can generate unpleasant situations like halitosis. Halitosis is a latin word which derived from halitus (breathed air) and the osis (pathologic alteration),and it is used to describe any disagreeable bad or unpleasant odor emanating from the mouth air and breath. Foetor oris, oral malodor, mouth odor, bad breath, and bad mouth odor are the other terms which are used to describe and characterize the halitosis. This undesirable condition is a common complaint for both genders and for all age groups. It creates social and psychological disadvantages for individuals, and these situations affect individual's relation with other people. In present review we describe the etiological factors, prevalence data,etiology, diagnosis, and the therapeutic mechanical and chemical approaches related to halitosis.

Prevalence: - Halitosis is very common in general population and nearly more than 50% of the general population have halitosis. In a Swedish study of 840 men, halitosis assessment was only present in around 2% of the population. However, halitosis prevalence in a China study which involved more than 2500 participants was assessed above 27.5%. Also in the literature, the prevalence of halitosis reported as ranging from 5% to 75% of tested children. Origin of halitosis in 90% of the patient is oral cavity; 9% of patient source of halitosis is non-oral reasons such as respiratory system, gastrointestinal system, or urinary system. In 1% of patients, the cause of the halitosis is diets or drugs.

Etiology of Halitosis: - Halitosis is formed by volatile molecules which are caused because of pathological or nonpathological reasons, and it originates from an oral or a non-oral source. These volatile compounds are sulfur compounds, aromatic compounds, nitrogen-containing compounds, amines, short-chain fattyacids, alcohols or phenyl compounds, aliphatic compounds, and ketones. Volatile sulfur compounds (VSCs) are mainly responsible for intra-oral halitosis. These compounds are mainly hydrogen sulfide and methyl mercaptan. They produce bacteria by enzymatic reactions of sulfur-containing amino acids which are L-cysteine and L-methionine.

Halitosis originates from oral cavity:- Although halitosis has multifactorial origins, the source of 90% cases is oral cavity. In oral cavity, temperatures may be reached up to 37°C (and changed between 34 and 37°C). During exhaling also humidity may be reached up to 96% (and changed between 91% and 96%) in oral exhalations. These conditions may provide a suitable environment for bacterial growth. There are more than 500 different bacterial species in the oral cavity, and the majority of them are able to create odorous substances that can lead to halitosis. In these circumstances, poor oral hygiene is a major contributor to the growth of the halitosis-causing bacteria and the subsequent rise in halitosis. These bacteria are primarily found in the tongue coating and periodontal pockets, and they are particularly Gr-negative species and proteolytic obligatory anaerobes. Some healthy people with no background of halitosis or periodontal disease will exhibit halitosis as a result of bacterial buildup on the surface of the tongue. These microorganisms create pungent chemicals by degrading organic materials (such as glucose, mucins, peptides, and proteins found in saliva, crevicular fluid, oral soft tissues, and retained detritus).

II. MATERIAL AND METHODS:

Eugenia caryophyllus (Clove) oil extraction:-

Extract of oil from flower bud is done using ethanol. The shade dried coarse powder of the buds (500gm) was filled in soxhlet apparatus and started continuous hot extraction with ethanol until the extraction completed. The obtained extract was filtered when it was hot and the obtained extract was distilled in vacuum under lower pressure as to remove the solvent completely.
Then it is dried and keep in a desiccator for experimentation. oil obtained was weighed and the practical yield and percentage yield was measured. After that the volatile oils is mixed with solution of 3% sodium or potassium hydroxide for eugenol extraction. This process leads to form alkali salt of phenol. Then by solvent extraction the insoluble part of extract is isolated.
The remained alkaline solution is acidified at refrigeration temperature, the removal of eugenol done by placing various methods such as fractional distillation, thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC

Trigonella foenum-graceum (Fenugreek) seed extraction:-

Fenugreek seed was purchased from gokuldas kalidas kirana shop of gondia city. The seed is cleaned and washed by water, dried in shade after that grinded using mixer grinder. The powder seeds then mixed with little ethanol and water which is distilled and sterlised. For microbial testing, 50 g of fenugreek seed powder was mixed with 500 ml of ethanol. Again in second beaker 50 g of the powder seed was added to 500 ml of distilled water. The two mixtures were placed in a rotary shaker for 24 hours and then using Whatman no.1 filter paper fitered it. Then 0.45 µm Micro filter was used in a rotary evaporator at 50°C for more filtration. The extract was stored at 4°C upto experimentation.

Evaluation of Drug Extract & Oil Analysis:-

- Clove *(Eugenia caryophyllus)*

✓ Colour: - Reddish Brown Odour: - Aromatic Taste: - Spicy
✓ Extractive values of *Eugenia caryophyllus* oil was found to be 4.6 % v/v in aqueous solvents.
✓ Extracts showed little fluorescence.
✓ It has given positive tests for phytosterols, flavonoids, triterpenes, alkaloids and carbohydrates.
✓ Extract was free from saponins, tannins, glycosides, amino Acids and proteins.
✓ Alcohol-soluble extractive :- 70.0% w/w
✓ Water-soluble extractive value :- 10.0 % w/w
✓ Fluorescence analysis :- No fluorescence

- Fenugreek seed *(Trigonella foenum-graceum)*

✓ Colour: - Yellow Odour: - Characteristics Taste: - Strongly Bitter
✓ It given positive tests for alkaloid, , carbohydrate, tannins flavonoid.
✓ Alcohol-soluble extractive:- 7.0% w/w
✓ Water-soluble extractive value:- 14.0 % w/w
✓ Fluorescence analysis:- No fluorescence

Antimicrobial Action of Clove and Fenugreek Extract

Microbicidal Test Well-diffusion pour plate method, this method is based on the drug extract release in cavities prepared on the solidified agar medium in petri plate, upto the level by which the growth of added microbes in agar plate get stopped, producing clearly visible circular zone of inhibiton in petri plate around the cavity filled with extracts. 0.2 ml of the test organism (S.aureous) was inoculated in the sterilized agar medium.then sterilized the cup-borer of 10 mm diameter by dipping it in alcohol containing beaker and then flaming it, then prepare four wells in the petri plate containing solid agar medium previously inoculated with microorganism by borer, one in each quadrant, at same distance. Pour different concentration of the drung extract in the holes prepared by borer, placed in Incubator at 37 degree Celsius for 24 hr, after it the zone of inhibition is checked and measured by scale.
Microorganism for Experimentation is: - Staphylococcus aureus (gram+ve) bacteria procured from department of microbiology Gondia college of pharmacy, Gondia.

Name of the chemicals Apparatus and equipments used in the experimentation:-

- ✓ Nutrient Agar (for bacteria growing)
- ✓ Dimethyl sulphoxide
- ✓ Incubator and laminar air flow
- ✓ Microorganism culture prepared within 12-15 hr
- ✓ Different drug extract concentratons
- ✓ Cork borer and Sterile petri plates

Agar plate method (Well-diffusion) :-

Fisrtly prepared fresh agar media and sterilised it using autoclave. 500$\mu.lit$.of microorganism inoculum added in 250 ml of media in laminar air flow (aseptic condition) and then the agar medium was poured in petriplates. After the medium get solidified, cavities were prepared using sterile sterile borer, pH was adjusted between 7.8 - 8.0.

Preparation of Sample:-

For preparing test sample of extract, Dimethyl sulphoxide solvent used for dissolution, in which 100 mg of drug extract was dissolved in 100 ml of Dimethyl sulphoxide solution the resulting solution then get filtered. Then this prepared sample was used for determining antimicrobial activity.

Different concentration of drug extract were prepared, range is 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg/ml, After that 4 cavities in every plate containing pre-solidified agar medium was prepared, there were total 6 plates prepared same as described above. In the cavity prepared on agar plate 0.3 ml of prepared sample of (eugenol) and on another group of 6 petri plates (fenugreek seed extract) concentrations are filled.

Concentrations are filled in cavities as conc. 0.1, 0.2, 0.3, respectively till 1.0 mg/ml in clockwise direction.

The antimicrobial activity of test organism for each extract was shown by clear zone of inhibition around the cavities.

Result is calculated by measuring diameter of zone of inhibition. For avoiding error in the result or for preventing faulty results the test control is employed there in the aseptic area, in aseptic room and in the incubator as well.

Chewing Gum Preparation:

Prolamin Extraction from Wheat:

100gm of accurately weighed quantity of Wheat (T.aestivum) flour is mixed with 300ml of, then it subjected to 2 hour stirring, After it the resulting liquid get strained with the help of multilayer muslin cloth for removing the extracted liquid from the wheat flour waste or residue. The extracted liquid is then heated at 50°C concentrated to get prolamin. To this concentrated liquid added equal amount of water and heated at 70°C until the solid gum base was formed.

Isolated Gum Base Evaluation:

The gum base was evaluated for swelling index and water retention capacity for determing the amount of water required for drug release. For determining the swelling index, 1gm of gum base powder (previously weighed) transferred to measuring cylinder of 100 ml, initial volume was recorded and distilled water was mixed. After gentle shaking, the measuring cylinder was placed aside for 24 hour at ambient humidity and temperature. The sedimented powder was seen at the bottom of measuring cylinder, volume of sediment is recorded. For determining the water retention capacity of gum base, the substance remained in the measuring cylinder during the experimentation of the swelling index was then filtered using muslin cloth.

The water was allowed to remove from it and volume was recorded. The initial volume of drained water is subtracted from the final volume of drained water this is water retension capacity.for finding loss on drying, 1gram of gum base weighed and heated in a hot air oven at 105℃ after that the gum weight was calculated, then loss on drying was calculated. Loss on drying is the difference between the initial weight of gum base and the final weight of gum base this expressed in percentage.

Preparation of Medicated Chewing Gum:

It was prepared by using extracted prolamin from the wheat, glycerin as plasticizer, calcium carbonate as texturing agent, sodium saccharin as artificial sweetener, polyvinyl acetate for incrasing elasticity of gum base, and peppermint oil as a flavoring agent. on the basis of different trails, the concentration of gum base , polyvinyl acetate, and calcium carbonate, was selected for the preparation of chewing gum. For calcium carbonate and gum base were 75 to 95mg and 455 to 545 mg respectively. Sticky or Hard solid gum was prepared, if deviated these ranges. Quantity Sufficient weighed of gum base, saccharin, and calcium carbonate, were mixed with the help of mortar and pestle. In small quantity of ethanol polyvinyl acetate was dissolved and mixed with the above excipients. After that obtained MIC concentrations of *Eugenia Caryophyllus* and *Triagonella foenum graceum* drug extract as 0.9 ml *Eugenia caryophyllus and* 1.0 ml of *Trigonella foenum-graceum* is mixed with the mixture, and at last peppermint oil and glycerin were mixed. This mixture was mixed until the solid dump mass of gum base was formed. By pressing the dump mass thin ribbon strip is made by hand and cetted it in the desired shape and size.

Anti microbial activityof chewing gum:

As previous experimentation of MIC for drug extract for chewing gum evaluation I have used well diffusion agar plate method, prepared agar medium poured it on plate, keep few minutes for solidification and prepared 6mm hole by using sterile borer and inoculated it with staphylococcus aureus gram+ve bacteria inoculums. Previously prepared chewing gum filled in the bored cavity and incubated for 24hour at 37 degree celsius and calculated the zone of inhibition around the cavity recorded.

III. RESULT:

From the obtained results both drug *Eugenia Caryophyllus* oil and *Trigonella foenum-graceum* seed extract in chewing gum showed desired antimicrobial activity for test microorganisms (Gram positive bacteria).The Minimum inhibitory concentration of chewing gum was found within the range of 4.8 – 5.5 mm, which concludes that it can treat Halitosis.
Antimicrobial activity: Maximum zone of inhibition for *Eugenia caryophyllus* oil observed was 4.0 mm at 0.9 ml dilution and *Trigonella foenum-graceum* seed extract observed was 8.0 mm at 1.0 ml dilution.
The Combination MIC of both drug was between 6.0 mm to 8.0mm at 0.9ml and1.0ml dilution.
The Minimum Inhibitory Concentration of blank was calculated is **0.00 mm** for the concentration of **1ml DMSO solution**.

Table 1:- *Eugenia caryophyllus* Antimicrobial Activity

Sr.no.	Concentration (ml)	Bacteria: gram positive Staphylococcus aureus (Radius)
1.	0.1 ml	-
2.	0.2 ml	1.5 mm
3.	0.3 ml	2.3 mm
4.	0.4 ml	3.0 mm
5.	0.5 ml	3.1 mm
6.	0.6 ml	3.6 mm
7.	0.7 ml	4.0 mm
8.	0.8 ml	4.0 mm
9.	0.9 ml	4.0 mm
10.	1.0 ml	3.9 mm

Table 2:- *Trigonella foenum-graceum* Antimicrobial activity

Sr.no.	Concentration (ml)	Bacteria: gram positive Staphylococcus aureus (Radius)
1.	0.1 ml	-
2.	0.2 /ml	1.0 mm
3.	0.3 /ml	1.0 mm
4.	0.4 /ml	3.0 mm
5.	0.5 /ml	4.0 mm
6.	0.6 /ml	6.0 mm
7.	0.7 /ml	6.5 mm
8.	0.8 /ml	7.3 mm
9.	0.9 /ml	8.0 mm
10.	1.0 /ml	8.0 mm

Table 3:- Antimicrobial activity of combinations of *Trigonella foenum-graceum* and *Eugenia Caryophyllus*

Sr.no.	Concentration (ml) of Both Drug	Zone of Inhibition (Radius)
1.	0.9 ml + 1.0 ml	6.5 mm
2.	0.9 ml + 1.0 ml	6.2 mm
3.	0.9 ml + 1.0 ml	7.0 mm
4.	0.9 ml + 1.0 ml	6.0 mm
5.	0.9 ml + 1.0 ml	6.2 mm
6.	0.9 ml + 1.0 ml	8.0 mm
7.	0.9 ml + 1.0 ml	6.0 mm
8.	0.9 ml + 1.0 ml	7.5 mm
9.	0.9 ml + 1.0 ml	8.0 mm
10.	0.9 ml + 1.0 ml	7.3 mm
11.	0.9 ml + 1.0 ml	6.0 mm
12.	0.9 ml + 1.0 ml	8.0 mm

Table 4:- Antimicrobial activity of chewing gum

Sr. No.	Chewing gum in cavity	Zone of Inhibition (Radius)
1.	C1	5.2 mm
2.	C2	5.5 mm
3.	C3	4.8 mm
4.	C4	5.1 mm
5.	C5	5.0 mm

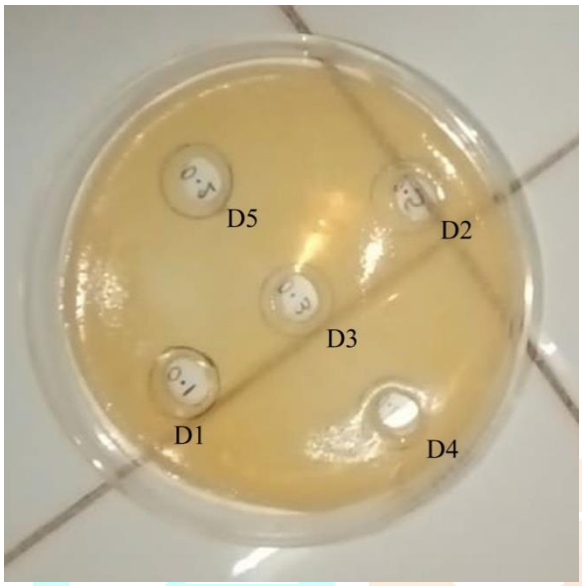


Fig. Antimicrobial Activity of Drug Combination

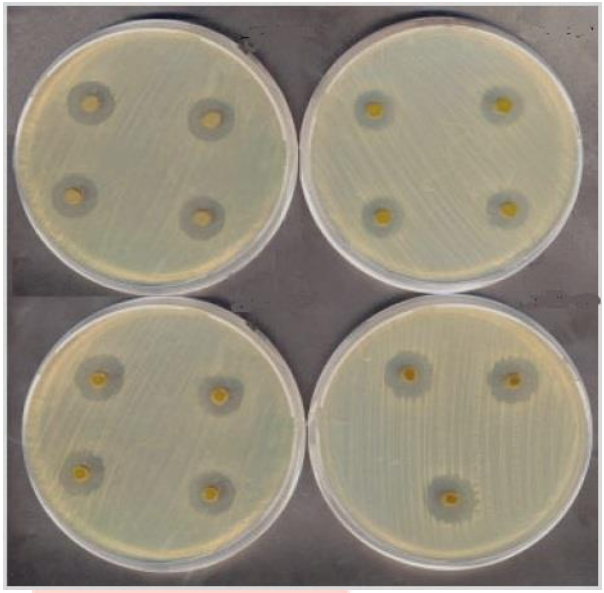
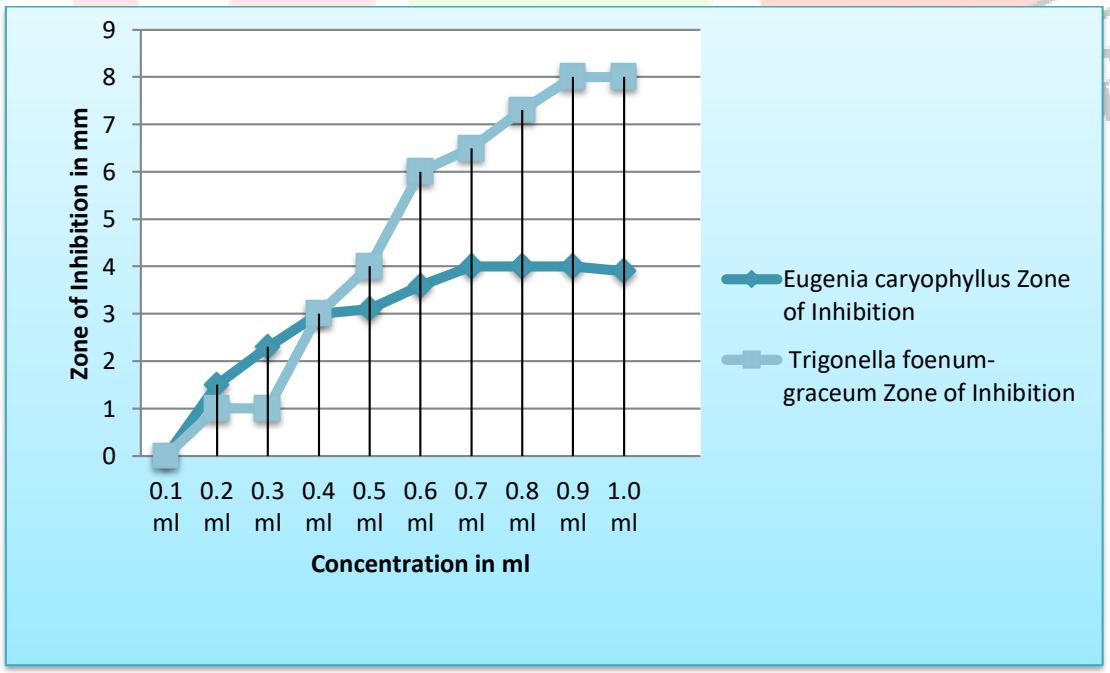


Fig. Antimicrobial Activity of Chewing gum

Antimicrobial Activity Chart :- 1



Evaluation Parameters of Medicated Chewing Gum:

Texture Evaluation:

Four results were obtained for texture evaluation which is solid mass, sticky, good to very good. Results of texture evaluation after performed for S1 to S9 are shown in below Table. Preparation S1, S2, and S8 showed good texture. Preparation S4 and S7 were observed to be sticky, and S3, S6, and S9 were hard to feel. Preparation S5 was observed that have very good texture.

Elasticity Study:

The elasticity of S1 to S9 Evaluated by CT3 texture analyzer S1–S3, S4–S6, and S7–S9 containing increase in level of calcium carbonate at same level of gum base shown elasticity reduced. Preparation S4, S5, and S7 were Observed to have highest elasticity. Preparation S1, S4, and S7 containing increase in level of gum base at same level of calcium carbonate observed elasticity incretion. S3, S6, and S9 which have increase level of gum base and calcium carbonate at high level showed elasticity reduction.

Preparation Name	Color	Texture	Elasticity testing (mm)
S1	yellow	Good	11.9
S2	yellow	Good	10.5
S3	yellow	Solid mass	10.22
S4	yellow	Sticky	12.65
S5	yellow	Very good	12.51
S6	yellow	Solid mass	7.9
S7	yellow	Sticky	12.77
S8	yellow	Good	10.29
S9	yellow	Solid mass	4.8

IV. DISCUSSION:

The present study is regarding antimicrobial activity of *Eugenia Caryphyllus* and *Trigonella foenum gracium* seed exrtract as we see current scenario of world if doctor say patient to use gargle for prevention of Halitosis or bad breath of it, the patient will not use it regularly as he sees it as a therapy but if formulate this chewing gum using these plant based chemicals which has no harmful effect on mouth cavity he sees as a fun by chewing it. So he will prefer chewing gum over the gargle preparation, so we can treat the halitosis by using just a simple preparation having antimicrobial effect on halitosis microorganisms which will kill them and prevent patient from the bad breath of mouth.

V. CONCLUSION:

Both the Drug eugenol from the clove and fenugreek seed extract given optimum antimicrobial activity on the test microorganism. Minimum inhibitory concentration found was 4.8 – 5.5 mm for bacteria *S. aureus* (gram positive bacteria)
Thus it is concluded that chewing gum preparation can be used to cure or prevent Halitosis. Which also prevent patient from bad breath, dental ache, gum swelling like problems by killing the causative microorganisms which are mainly gram positive bacteria.

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