FORMULATION AND EVALUATION OF POLYHERBAL LOZENGES TO TREAT TONSILLITIS

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ABSTRACT

Tonsillitis is an inflammation of the pharyngeal tonsils commonly caused by *Streptococcus pyogenes* and other microbial infections. Prolonged use of antibiotics may lead to resistance and side effects, highlighting the need for safer herbal alternatives. The present study aimed to formulate and evaluate polyherbal lozenges using *Justicia adhatoda*, *Piper betle*, and *Trachyspermum ammi* for the symptomatic relief of tonsillitis. The crude powders were subjected to macroscopic, microscopic, physicochemical, and preliminary phytochemical analysis. The extracts showed the presence of alkaloids, flavonoids, tannins, carbohydrates, saponins, and glycosides. Polyherbal lozenges were prepared by the moulding method and evaluated for physical characteristics, pH, weight variation, and friability. Anti-inflammatory activity was assessed using the albumin denaturation assay. The lozenges showed significant inhibition (83.10% at 100 µg/ml) with an IC_{50} value of 60.20 µg/ml, compared to aspirin. Antimicrobial activity was evaluated by agar well diffusion method against *Streptococcus pyogenes* and *Escherichia coli*. The lozenges demonstrated strong antimicrobial activity with zones of inhibition comparable to the standard drug. The study concludes that the formulated polyherbal lozenges possess promising anti-inflammatory and antimicrobial properties and may serve as an effective herbal remedy for tonsillitis.

INTRODUCTION:

Tonsillitis is inflammation of the pharyngeal tonsils. The inflammation usually extends to the adenoid and the lingual tonsils; therefore, the term pharyngitis may also be used. Most cases of bacterial tonsillitis are caused by group A beta hemolytic Streptococcus pyogenes (GABHS). Pharyngotonsillitis and adeno-tonsillitis are considered comparable for the purposes of this article. Lingual tonsillitis states to isolated inflammation of the lymphoid tissue at the tongue base. Tonsillitis most frequently occurs in children; nevertheless, the condition infrequently occurs in children younger than 2 years.[1]

TYPES OF TONSILLITIS:

- Acute tonsillitis-caused by bacterial/viral.
- Recurrent tonsillitis-caused by bacterium actinomyces.
- Chronic tonsillitis-caused by bacterial infection.
- Peritonsillar abscess-caused by bacterial/viral.[2]

Symptoms of tonsillitis are mainly dry Throat, sore throat, dry cough or pharyngeal foreign body sensation.[3] Now a day, prolonged use of antibiotics can cause side effects and drug resistance. Studies found that medicinal plants are now effective to treat tonsillitis. Ancient Siddha studies denote *Adhathoda vasica* is used to treat tonsillitis.

In this study, Herbal Lozenges are made from *Adhathoda Vasica* and *piper betel* leaf, To treat symptomatic relief from Tonsilitis.

PLANT PROFILE

ADHATODA VASICA

SCIENTIFIC NAME : *Justicia adhatoda*

FAMILY **:** Acanthaceae

COMMON NAME **:** Malabar nut, adulsa, adhatoda, vasa, vasaka.

TAXNOMICAL CLASSIFICATION (integrated taxnomical information system) :

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnolipsida
Order : Lamiales
Family : Acanthaceae
Genus : Justicia
Species : Justicia Adhatoda

PIPER BETLE

SCIENTIFIC NAME ***:*** *Piper Betel*

FAMILY **:** Piperaceae

COMMON NAMES **:** Betel leaf , Betel nut

TAXNOMICAL CLASSIFICATION (integrated taxnomical information system) :

Kingdom : Plantae
Division : Magnoliphyta
Class : Magnolipsida
Order : Piperales
Family : Piperaceae
Genus : Piper
Species : P.betel

AJWAIN:

SCIENTIFIC NAME : *Trachyspermum ammi*
FAMILY : Apiaceae
COMMON NAME : Carom seed
Bishops weed
Ajowan caraway
Thymol Seed

TAXNOMICAL CLASSIFICATION (integrated taxnomical information system) :

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnolipsida
Order : Apiales
Family : Apiaeceae
Genus : Trachyspermum
Species : T. Ammi

MATERIALS AND METHODS

Collection of plant material:

The Crude powder drug of *Adhatoda vasica* and *Piper betle* was purchased from siddha pharmacy (Vijay siddha clinic, Savitha bus stand road, Vasuki 6th street, near universal hospital, sun agencies, erode fort, erode, Tamil Nadu – 638001)

Macroscopical Evaluation:

Leaves are studied separately for its morphological characters by organoleptic test like colour, odour, size, shape and taste etc.

Powder microscopy:

Taken a clean slide and stain the powder using phloroglucinol and conv. HCL. Powder microscopy was observed under Compound microscope and image was clearly taken by phone.

QUALITATIVE ANALYSIS:[4]

Determination of ash value: The ash remaining after complete ignition of the medicinal plant materials is determined by three different methods known as Total ash, Acid insoluble ash and water-soluble ash. An accurately weighed 2g of air dried coarsely powdered drug was taken in a tarred silica crucible and incinerated in muffle furnace at a temperature not exceeding 450°c, until free from carbon then allowed to cool and weighed. The percentage of ash was calculated with reference to the air dried drug.

Determination of moisture content by loss on drying: Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead tithe activation of enzymes and gives suitable conditions to the proliferation of living organism. As most vegetable drugs contain all the essential food requirements for mould, insects and mites, deterioration can be very rapid, once infestation has taken place. Various methods for moisture determination are loss on drying, separation and measurement of moisture, chemical methods, electrometric methods, and spectroscopic methods as per IP. 10gm of powder was weighed and placed it in a moisture content apparatus. Temperature was adjusted to 100-110^{0} c till weight get constant and collected in desiccators and weighed. The loss of weight was regarded as a measure of moisture content as per IP.

Determination of extractive value: About 5g of the air dried coarse powder of leaves of adhatoda vasica was macerated with 100ml of hexane, chloroform, petroleum ether, ethyl acetate, ethanol and water separately in the closed flask for 24 hours. The flask was shaken frequently during the 1st 6 hours and was allowed to stand for 18 hours. There after it was filtered rapidly, Taking precaution against loss of the solvent. About 25ml of filtrate was evaporated to dryness at 105c in the tarred flat- bottomed shallow dish and weighted. The percentage of ethanol soluble extractive was calculated with reference to their air dried drug.

PRELIMINARY PHYTOCHEMICAL SCREENING

Hydro-alcoholic extract subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, amino acids, and sterols as per the procedure.[5]

TEST FOR ALKALOIDS: About 2 gm of the powdered material is mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200mL of chloroform was added, mixed well and refluxed for half an hour on water bath. Then it was filtered and the chloroform was evaporated. To this 5mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

Drangendroff test: A small quantity of the extract is treated with Drangendroff reagent. Pale yellow color indicates the presence of alkaloids.
Mayer test: A small quantity of the extract is treated with Mayer's reagent. Pale yellow color indicates the presence of alkaloids.
Wagner test: A small quantity of extract is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.
Hager's test: A small quantity of extract is treated with Hager's reagent. Yellow color precipitate indicates the presence of alkaloids.

TEST FOR CARBOHYDRATES:

Molisch's test: The extract of the powdered drug is treated with 2-3 drops of 1% alcoholic α naphthol and 2mL of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

Fehling's test: The extract of the powdered leaf is treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

Benedict's test: The extract of the powdered leaf is treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

TEST FOR FLAVONOIDS:

Shinoda's test: Little of the powdered drug is heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour is obtained indicating the presence of flavonoids.

Alkali test To the small quantity of test solution 10% aqueous sodium hydroxide solution is added. Yellow /orange colour is produced indicating the presence of flavonoids.

Lead acetate: To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white precipitate.

TEST FOR ACID

To the small quantity of test solution, few drops of concentrated sulphuric acid are added. Yellow orange colour is obtained indicates the presence of flavonoids.

TEST FOR TANNINS:

Ferric chloride test: A small quantity of the powdered drug was extracted with water. To the aqueous extract, few drops of ferric chloride solution were added. A bluish black colour indicates the presence of tannins.

Lead acetate test: To 1 ml of extract, 1 ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

TEST FOR PROTEINS AND FREE AMINO ACIDS:

Xanthoproteic test: 1 ml of extract was added 2 – 6 drops in concentrated HNO2 this solution was neutralized with alkali. Protein solution yellow or orange colour. Protein solution shows the presence of protein.

Biuret test: To the extract of the powdered leaf 1 ml of dilute solution of sodium hydroxide was added. A violet colour will be obtained which indicates the presence of proteins.

TEST FOR ANTHRAQUINONE GLYCOSIDES:

Bontrager's test: The powdered drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammonical layer showing the presence of anthraquinone glycosides.

Modified Bontrager's test: About 0.1 g of the powdered drug is boiled for 2 minutes with dil. HCl and few drops of FeCl3 solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil.NH3 solution was added to the benzene extract. No pink color was observed in ammonical layer showing the presence of glycosides.

FORMULATION OF POLYHERBAL LOZENGES:

- Clean and dry all the apparatus required for preparation
- Accurately weigh lactose (0.3 g), gelatin (0.3 g), mannitol (2 g), sucrose (1.5 g), magnesium stearate (0.07 g), citric acid (0.08 g), and plant extracts (0.2 g). Keep ajwain oil (1drop) separately.
- Take a small quantity of water in a beaker and heat gently on a water bath.
- Add gelatin slowly to the warm water with continuous stirring until a clear gelatin solution is obtained.
- In another beaker, dissolve sucrose and mannitol in warm water to prepare a sugar syrup.
- Add the sugar syrup gradually into the gelatin solution with constant stirring to obtain a uniform mixture.
- Add lactose, citric acid, and plant extracts to the above mixture and stir continuously to ensure uniform distribution.
- Add one drop of ajwain oil and mix well.
- Add magnesium stearate as a lubricant and mix gently.
- Pour the warm prepared mass into previously lubricated lozenge molds.
- Allow the molds to stand at room temperature until the lozenges solidify
- Remove the solidified lozenges from the molds and store them in a well-closed container.

S.NO	INGREDIENTS	QUANTITY	S.NO	INGREDIENTS	QUANTITY
1	Lactose	0.3g	7	Ajwain oil	1 drop
2	Gelatin	0.3g	8	Plant Extracts	0.2g
3	Mannitol	2g	9	water	qs
4	Sucrose	1.5g			
5	Magnesium citrate	0.07g			
6	Citric acid	0.08g			

EVALUATION OF POLYHERBAL LOZENGES:

Physical Examination of lozenges:

All the formulated patches were evaluated visually for appearance in terms of colour, odour and taste.

pH:

Take a specific amount of the lozenge (usually 1-2 lozenges) and dissolve it in a known volume of distilled water (for example, 100 mL). Stir until the lozenge is completely dissolved.The pH value ranges between 5.7– 6.9.

Weight variation:

The weight variation test is done by weighing 5 lozenges individually and then by taking average comparing it as follows:

Weight variation = (average weight – initial weight) ÷ average weight

Friability:

Friability testing is carried out by using Roche Friabilator operated at specific speed for specific time such 25 rpm for 4 min.

Friability (%) = (W1 - W2) / W1 × 100

where - W1 = Initial weight of tablets; W2 = Final weight of tablets after tumbling***.(6)***

ANTI-INFLAMMATORY ASSAY OF POLYHERBAL-LOZENGES ALBUMIN DENATURATION

ALBUMIN DENATURATION ASSAY

The albumin denaturation method is widely used for screening anti-inflammatory properties because inflammation is often associated with protein denaturation. The ability of a substance to prevent or inhibit albumin denaturation suggests its potential anti-inflammatory activity. ***(7)***

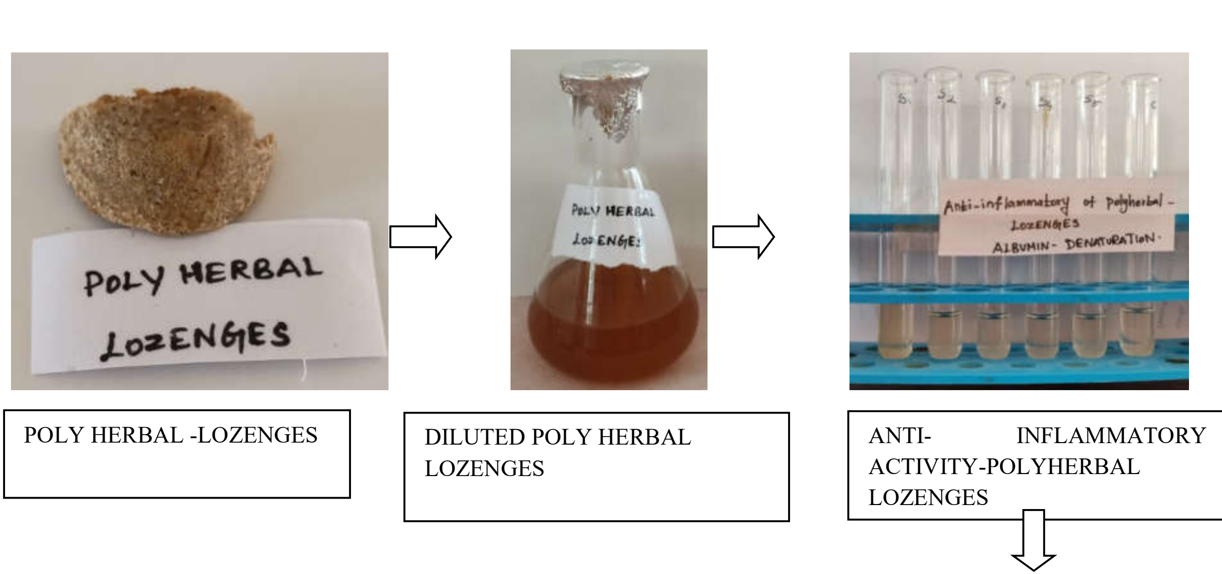
Materials:

- Bovine serum albumin (BSA) (Sigma-Aldrich, Catalog No. A3059)
- Phosphate buffer saline (PBS)
- Test samples (plant extract, drug, or natural compound)
- Heat source (water bath or incubator)
- Distilled water

Method:

1. Prepare a solution of bovine serum albumin (BSA) in PBS (1% w/v).
2. Prepare the test sample (plant extract, synthetic compound, etc.) at varying concentrations.
3. Add the test sample to the BSA solution and incubate for 15 minutes at 25°C.
4. After incubation, heat the solution to 70°C for 15 minutes to induce denaturation.
5. The test sample's ability to prevent albumin denaturation is assessed by measuring the absorbance at 660 nm or through visual inspection for precipitate formation. Lower absorbance or absence of precipitate indicates anti-inflammatory activity.

$$\text{Inhibition Percentage} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$



POLY HERBAL -LOZENGES

DILUTED POLY HERBAL LOZENGES

ANTI- INFLAMMATORY ACTIVITY-POLYHERBAL LOZENGES

The percentage inhibition of protein denaturation was calculated as

Percentage inhibition=

(Absorbance of control−Absorbance of sample)×

100/ Absorbance of control

Measured absorbance AT 660 nm by UV Visible spectrophotometer

ANTIMICROBIAL ACTIVITY OF POLYHERBAL LOZENGES

TEST MICROORGANISMS:

Totally Two bacterial strains were used throughout investigation. All the bacterial and fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, and Chandigarh, India. The bacteria used were *Streptococcus pyogenes* and *E. coli*. The young bacterial broth cultures were prepared before the screening procedure.

ANTIBACTERIAL ASSAY

I. Preparation of inoculums: Stock cultures were maintained at 4ºC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37ºC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 X 10^6 colony forming units (CFU/ml) for bacteria. ***(8)***

II. Preparation of sterile swabs: Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes, papers, or tins etc.

III. Sterilization of forceps: Forceps can be sterilized by dipping in alcohol and burning off the alcohol.

ANTIBACTERIAL ASSAY USING AGAR WELL DIFFUSION METHOD

The well diffusion method was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates could solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly, and the inoculums could dry for 5 minutes. Wells were cut and 20 µl of the different concentration of test drug were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

RESULT AND DISCUSSION:

Table No: 1 - Macroscopic characters of *Adhatoda Vasica* and *Piper betle*

MACROSCOPIC CHARACTERS		*Adhatoda Vasica*	MACROSCOPIC CHARACTERS	*Piper betle*
Apex	Acuminate		Acuminate	
Base	Acute		Obtuse	
Margin	Entire margin		Entire margin	
Vein	Reticulate vein		Reticulate vein	

Table No: 2 - Powder microscopy OF *Adathoda vasica* & *Piper betle*:

CHARACTERISTICS	***ADATHODA VASICA***	CHARACTERISTICS	***PIPER BETLE***
FIBER		FIBRE	
STOMATA		SPIRAL SHAPED XYLEM VESSEL	
TRICHOMES		MUCUS CANAL	

PHYSIO-CHEMICAL STUDIES:

Table No: 3- Physiochemical constants of leaf powder:

S.No	Physiochemical Constants	*Adhatoda vasica*	*Piper betle*
1	**Total Ash Value**	6%	17%
2	**Loss on Drying**	15.5%	4%
3	**Aqueous Extract**	1.56%	0.73%
4	**Ethanol Extract**	0.3%	0.33%
5	**Petroleum Ether Extract**	0.13%	0.06%
6	**Ethyl Acetate Extract**	0.1%	0.16%
7	**Hexane Extract**	0.16%	0.03%
8	**Chloroform Extract**	0.16%	0.13%

PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDROALCHOLIC EXTRACT OF *ADHATODA VASICA* AND *PIPER BETLE*:

Hydroalcholic extract of adhatoda vasica and piper betle were subjected to both qualitative chemical analysis. The various chemical test were performed for both the extracts for the identification of phytoconstituents. The results were displayed in below table:

Table No: 4 - Phytochemical screening of *Adhatoda vasica*

S.NO	PHYTOCHEMICAL SCREENING	Adhathoda vasica	Piper betle
1	**Test for Alkaloids**		
	Mayer's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
2	**Test for Carbohydrates**		
	Molish's test	+	+
	Fehling's test	+	+
3	**Test for flavonoids**		
	Shinoda Test	+	+
4	**Test for tannins**		
	Ferric chloride test	+	+
	Lead acetate test	+	+
5	**Test for saponins**		
	Foam test	+	+
6	**Test for mucilage**		
	Ruthenium red	+	+
7	**Test for anthraquinone glycosides**		
	Borntrager's test	+	+
	Modified borntrager's test	+	+

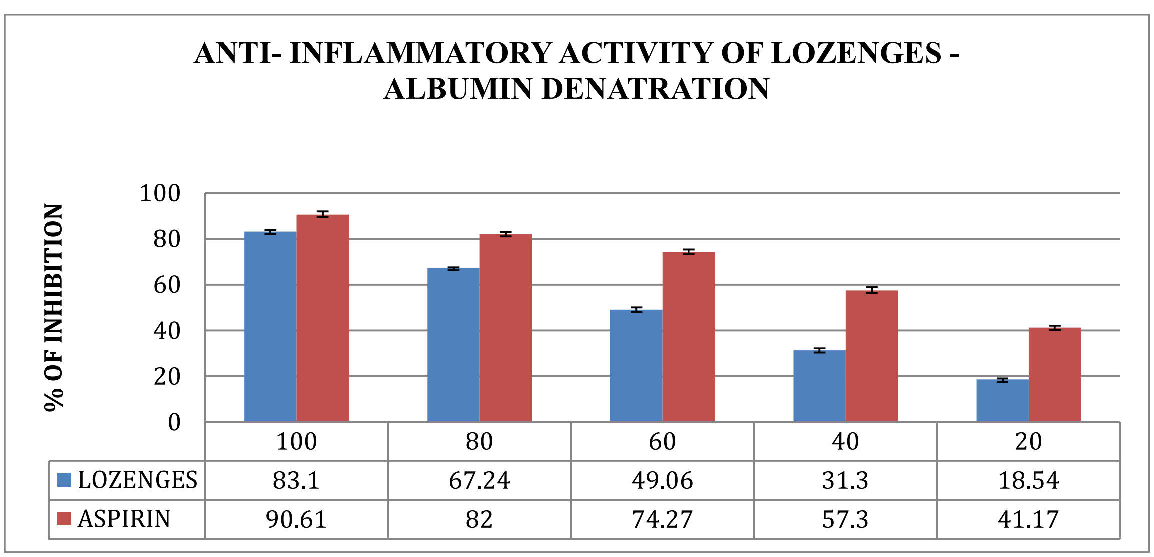
FORMULATION AND EVALUATION OF POLY HERBAL LOZENGES:

Fig 1: – Poly herbal lozenges

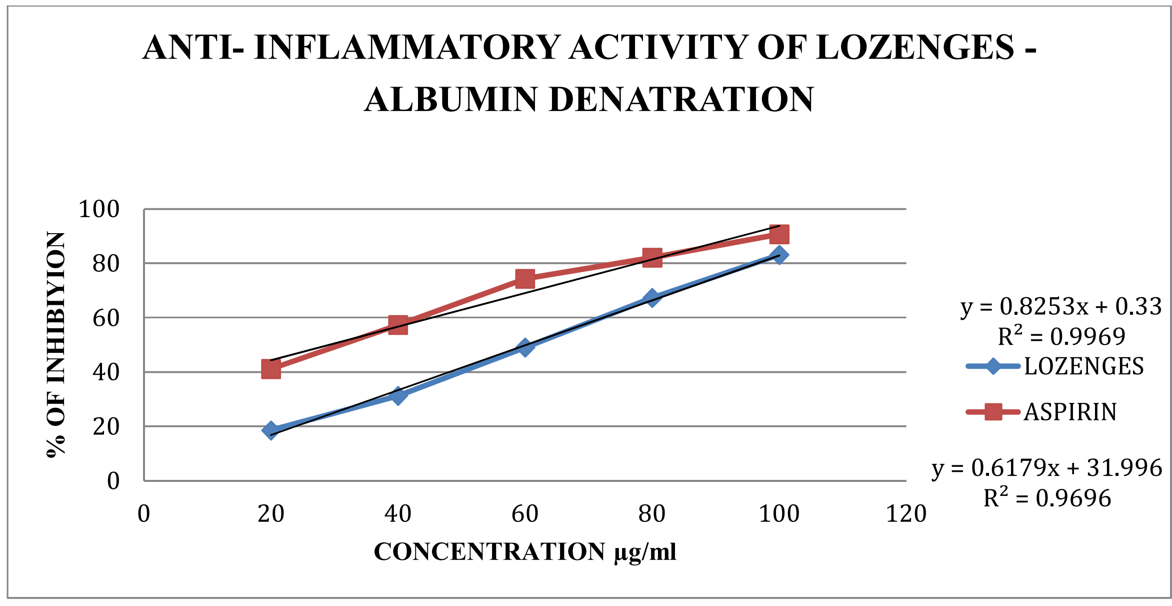
S.No	Characters	Characters of lozenges
1	Colour	Dark brown
2	Odour	Strong
3	Taste	Sweet

Table No :5 – ANTI-INFLAMMATORY ACTIVITY OF LOZENGES:

SL NO.	NAME OF DRUG	TEST CONC (µg/ml)	ALBUMIN DENATURATION	IC50 values
1	LOZENGES	100	83.10 ± 0.73	60.20(µg/ml)
		80	67.24 ± 0.19	
		60	49.06 ± 1.02	
		40	31.30 ± 0.90	
		20	18.54 ±0.48	
2	ASPIRIN	100	90.61 ±1.32	29.18(µg/ml)
		80	82.00 ±0.94	
		60	74.27 ± 1.03	
		40	57.30 ± 1.52	
		20	41.17± 0.85	



Anti- inflammatory activity of lozenges - albumin denatration

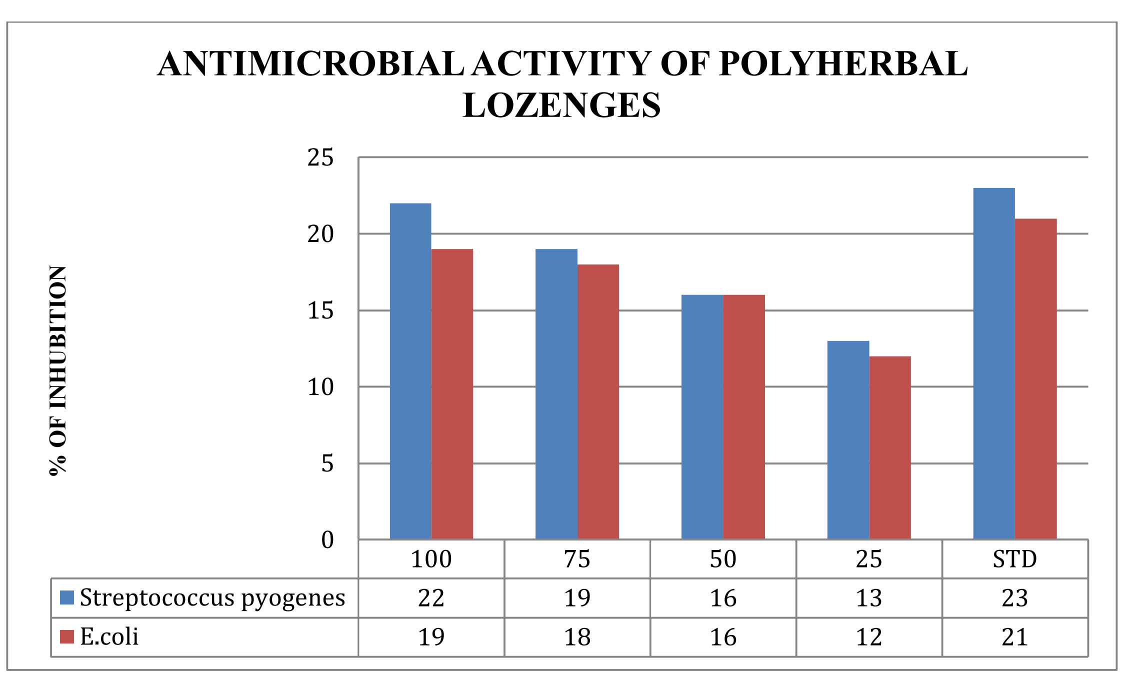


Graph No: 1 - Anti- inflammatory activity of lozenges - albumin denatration

Protein denaturation is a well-known cause of inflammation, and its inhibition is often linked to anti-inflammatory activity. In this study, the ability of different plant extracts to inhibit heat-induced albumin denaturation was evaluated Lozenges exhibited significant inhibition, with a maximum inhibition of Aspirin, a standard anti-inflammatory drug, showed maximum inhibition values of 90.61% and 83.10 % at a concentration of 50µg/ml. The IC50 values for Aspirin and Lozenges were found to be 60.20µg/ml and 29.18µg/ml respectively, indicating a moderate anti-inflammatory potential**.**

Table No:6 - ANTIMICROBIAL ACTIVITY OF POLYHERBAL LOZENGES

S. NO	MICROORGANISMS	ZONE OF INHIBITION (MM)				
		100	75	50	25	+ VE CONTROL
GRAM POSITIVE BACTERIA						
1	*Streptococcus pyogenes*	22	19	16	13	23
GRAM NEGATIVE BACTERIA						
5	*E.coli*	19	18	16	12	21



Antimicrobial activity of polyherbal lozenges

GRAM NEGATIVE

Fig 2: - Antimicrobial activity of polyherbal lozenges

GRAM POSITIVE

Fig 3: - Antimicrobial activity of polyherbal lozenges

The poly herbal lozenges concentration (100%) is likely equal to positive control. The zone of inhibition, that is positive control 23 & 21. In our poly herbal lozenges 22 and 19 (Streptoccous pyogenes and E coli). This shows our polyherbal lozenges having strong anti-microbial activity which helps to treat Tonsilitis.

CONCLUSION:

The present study focused on the formulation and evaluation of polyherbal lozenges incorporating *Adhatoda vasica* and *Piper betle* for the treatment of tonsillitis. The phytochemical screening revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, and saponins, which are known to possess antimicrobial and anti-inflammatory properties. The physio chemical analysis confirmed the purity and quality of the plant extracts, while powder microscopy facilitated the identification of key anatomical features. The anti-microbial assay demonstrated that the polyherbal lozenges exhibited significant inhibitory activity against both Streptococcus pyogenes and E. coli, indicating their potential as an effective remedy for bacterial infections associated with tonsillitis. Furthermore, the albumin denaturation assay highlighted the anti-inflammatory properties of the lozenges, further validating their therapeutic potential and symptomatic relief. Standardization of the lozenges, including physiochemical parameters, ensured consistency and quality of the product. The findings suggest that the combination of *Adhatoda vasica* and *Piper betle* in lozenge form could serve as a natural and effective alternative to synthetic drugs for managing tonsillitis and related infections. Future studies involving clinical trials and advanced pharmacological investigations are recommended to further substantiate the safety, efficacy, and mechanisms of action of these polyherbal lozenges. The research contributes to the growing field of herbal-based therapeutics, offering promising insights into the development of natural remedies for common infections.

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