JPharmSci
Journal of Pharmaceutical Sciences
Open access peer-reviewed Journal
www.jpsjournal.com [ISSN: 1520-601x]
JPHARMSCI

Development And Evaluation of a Polyherbal Formulation for the Treatment of Sore Throat

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ABSTRACT

Pharyngitis (sore throat) is the most common cause that brings patients to the outpatient and emergency departments all over the world, particularly children and adolescents. The aim of study was the development and evaluation of a polyherbal formulation for the treatment of sore throat. The leaves of Clitoria ternatea, Black pepper (seeds), Glycyrrhiza glabra (root), and Tulsi (leaves), were obtained from the Kanpur, UP. These were identified and authenticated by a botanist, separately. The plant materials were washed making dust-free and dried in shade at room temp. The leaves of Clitoria ternatea and Tulsi were soaked in hydroalcoholic solution (1:1) separately for 15 days with gradual stirrings. However, Black pepper (seeds), and Glycyrrhiza glabra (root) were extracted out through Soxhlet apparatus using ethanol, separately. Evaluation was done through parameters as i.e., organoleptic properties, weight uniformity, disintegration time studies, friability, measurement of ph, thickness, moisture, diameter and hardness. Evaluation of polyherbal formulation was done for the treatment of sore throat i.e., antimicrobial activity, bovine serum albumin denaturation assay and egg albumin denaturation assay. In conclusion, polyherbal lozenges of Clitoria ternatea (leaves), Black pepper (seeds), Glycyrrhiza glabra (root), and Tulsi (leaves) exhibited the antimicrobial potential when tested against the S. mutans and S. aureus microbial strains. It also demonstrated significant anti-inflammatory activity through protein denaturation. Therefore, the polyherbal lozenges of Clitoria ternatea (leaves), Black pepper (seeds), Glycyrrhiza glabra (root), and Tulsi (leaves) can be utilized in the management of pain and inflammation.

Keywords: Sore throat, polyherbal formulation, Ocimum sanctum, Glycyrrhiza glabra, lozenges.

INTRODUCTION

Around the world, pharyngitis, or sore throat, is the most frequent reason why patients, especially children and teenagers, visit outpatient and emergency rooms (Shulman et al., 2012). It covers pharyngeal mucosal inflammation with a variety of etiologies, including bacterial, fungal, viral, and non-infectious causes such environmental irritants and allergies. Even though viruses are the most frequent cause of illness, bacterial infections are more serious since they can lead to consequences if left untreated (Bisno et al., 2002). The prevalence of bacterial pharyngitis and its impact on both individual and public health make it a clinical issue. If left untreated, it can lead to non-suppurative consequences like glomerulonephritis and severe rheumatic fever as well as suppurative ones like peritonsillar abscesses (Gerber et al., 2009). The epidemiology of bacterial pharyngitis varies by age, region, and season. It is most common in late winter and early spring in temperate regions. Children of school age are the primary group implicated in transmission, and schools offer the close contact required for easy dissemination (Pfoh et al., 2008).

Plant profile

Aparajita (*Clitoria ternatea)*

The twining plant *Clitoria ternatea* grows in four different countries every year: India, China, the Philippines, and Madagascar. It belongs to the family Fabaceae. Flower is the aesthetically pleasing portion of the plant. These are available in single or paired configurations in several hues, including mauve, white, and both dark and light blue. The pedicles and bracteoles can reach lengths of 4-9 mm and 12 m, respectively, while the corolla has one regular petal,

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

two keels, and two wing petals. The standard petal is the biggest among all the petals (Kazuma et al. 2003). The flower is generally bilateral; nevertheless, petal mutations exist in nature, resulting in a radial appearance of the bloom. The leaves have pinnate venation with seven leaflets. The terminal leaflet is larger, while the base leaflet is smaller. The pods are flat, linear, and exhibit olive, brown, and black hues, measuring around 5-7 cm in length and holding 6-10 seeds per pod (Oguis et al. 2019). It contains the alkaloids, glycosides, flavonoids, resin, saponins, phenols, triterpenes, proteins, and carbohydrates.

Black pepper *(Piper nigrum)*

Black peppers, a foodstuff in the Piperaceae family of bulb-shaped plants, are arguably nature's most powerful meal (Ayurvedic Pharmacopoeia, 1989). The pepper plant's berries are the source of black pepper. In reality, black pepper, green pepper, and white peppercorns are all the same fruit (Piper nigrum); the color differences are due to different phases of maturation and processing techniques. When the pepper berries are half-ripe and on the verge of turning red, they are picked to make black peppercorns. After that, they are allowed to dry, which makes them shrivel and turn dark. On the other hand, white peppercorns are picked when they are extremely ripe and then soaked in brine to remove their dark outer shell, leaving only the white pepper seed6, whereas green peppercorns are picked while still unripe and green in color. In reality, pink peppercorns come from a different plant species called Schinus molle, which is related to ragweed. Of all the pepper varieties, black pepper is the strongest and most tasty. It can be purchased as whole or broken peppercorns or processed into powder (Umarkar et al. 2011). Pepper contains the alkaloids like piperine (5-9%), Volatile oil (1-2.5%), is responsible for the aroma of pepper and comprises of terpenes, like α- and ß

pinene, dipentene, Phenllandrene and sesquiterpenes. , pungent resin (6%), Piperidine and starch (30%). The pepper volatile oil which is yellowish in colour contains mainly L- Phenllandrene and caryophyllene. The resinous content along piperine also contributes to the pungency of the drug (Umarkar et al. 2011).

***Glycyrrhiza glabra* (Mulethi)**

The ancient Greek words glykas, which means sweet, and rhiza, which means root, are the sources of the word glycyrrhiza (Joshi and Tiwari, 2024). The plant is a herbaceous perennial that reaches a height of one to two meters and has a long, robust main taproot. The taproot is 15 cm long and has three to five offshoot taproots. New stems are produced every year. The leaves are alternating, drooping, and pinnately unique. The inflorescence resembles an erect spike and is 10–15 cm long. The blooms are bluish to pale violet in color and have a short pedicle that is 1-1.5 cm long. The fruit is a 1.5–2.5 cm long pod with a tiny, bell-shaped calyx. Three to five brown reniform seeds (Kaur et al. 2013). The roots of Glycyrrhiza glabra Linn contain a saponin called glycyrrhizin, which is 60 times sweeter than cane sugar and rich in flavonoids. Four new isoprenoid-substituted phenolic constituents—licoriphenone, 1-methoxyficifolinol, isoangustone A, and semilicoisoflavone B—were isolated from roots. Liquorice contains proteins, amino acids, polysaccharides, simple sugars, and mineral salts (such as calcium, phosphorus, sodium, potassium, iron, and others). magnesium, silicon, selenium, manganese, zinc, copper, pectin, resins, starches, sterols, and gums). Oestrogens, tannins, glycosides, coumarins, phytosterols (stigmasterol and sitosterol), vitamins (B1, B2, B3, B5, E, and C), and oestrogens have all been reported (Sharma and Vigyana, 2009).

Tulsi *(Ocimum tenuiflorum*)

Tulsi is one of the most important plants in Ayurveda. Tulsi is the finest Ayurvedic knowledge tonic for the body and mind, and it may treat a variety of contemporary disorders. It is a key component in the conventional "Ayurvedic" and "Unani" systems. One species or variant of the Lamiaceae family of plants is Ocimum tenuiflorum L. It has contributed to science from ancient times to the present day in current practices because of its many restorative potentials (Cohen, 2014).

Tulsi is an upright, aromatic plant with many branches. 30 to 60 cm tall, fragrant plant. The oval leaves have a basic green or purple color, a gently serrated or dented edge, and a blade length of 5 cm. The blooms have a short, hairy stalk and are violet.

The plant produces reddish-yellow seeds and little fruit. It has a harsh and caustic taste (Prajapati et al. 2003). Molludistin, orientin, luteolin, apigenin-7-O-glucuronide, ursolic acid, and luteolin-7-Oglucuronide are some of the additional compounds found in Tulsi leaf extract. This species of *Ocimum* also contains a number of monoterpenes and sesquiterpenes, such as bornyl acetate, β elemene, neral, pinenes (α and β), camphene, sitosterol, cholesterol, campesterol, and stigmasterol. The five types of fatty acids found in O. sanctum are stearic, palmitic, oleic, linoleic, and linolenic acid. Tulasi is rich in vitamin C, beta carotene, and calcium ions. In addition to tannins, camphor, and flavonoids such luteolin, orientin, vicenin, and triterpene; urolic acid; zinc, manganese, and sodium ions are all found in plant extracts (Singh S. et al. 1996).

MATERIALS AND METHODS

Experimental requirements

Fresh leaves of *Clitoria ternatea*, Black pepper (seeds), *Glycyrrhiza glabra* (root), Tulsi (leaves), Soxhlet apparatus, distilled water, rotatory evaporator, weighing machine and ethanol.

Collection, Identification, and extraction of plant

The leaves of *Clitoria ternatea,* Black pepper (seeds), *Glycyrrhiza glabra* (root), and Tulsi (leaves), were obtained from the Kanpur, UP. These were identified and authenticated by a botanist, separately. The plant materials were washed making dust-free and dried in shade at room temp. The leaves of *Clitoria ternatea* and Tulsi were soaked in hydroalcoholic solution (1:1) separately for 15 days with gradual stirrings. After 15 days, it is filtered with cotton plug and then finally with whatman filter paper to get the final extract. However, Black pepper (seeds), and *Glycyrrhiza glabra* (root) were extracted out through Soxhlet apparatus using ethanol, separately. It is filtered with cotton plug and then finally with whatman filter paper to get the final extract. The obtained slurry of mixture will be dried by a rotary evaporator or water-bath (Khan et al. 2020). The % yield of the different herbal extracts will be calculated using below mentioned formula-

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Preliminary phytochemicals determination (Khandelwal, 2002; Bhatt and Dhyani, 2012).

Estimation of alkaloids

Mayer's Test: The filtrates were treated with Mayer's reagent, also known as potassium mercuric iodide. When a yellow-colored precipitate appears, alkaloids are present.

Wagner's Test: The filtrates were treated with Wagner's reagent, which is potassium iodide mixed with iodine. When a brown or reddish precipitate appears, alkaloids are present.

Hager's Test: The filtrates underwent treatment with Hager's Reagent. Alkaloids may be present if yellow precipitation starts to develop.

Estimation of glycosides

Fehling's test: Fehling's solutions A and B were heated for one minute using distilled water dilution. This clear blue solution had eight drops of plant extract added to it. After that, it was mixed with one milliliter of Fehling's solution and cooked in a water bath for five minutes. The presence of glycosides is indicated by brick-red precipitation.

Estimation of saponins

Foam test: About 2g of the plant extract was mixed with 10ml of distilled water and agitated briskly to create a stable, long-lasting foam. The presence of froth is indicative of saponins.

Estimation of tannins

Ferric chloride test: A test tube containing 0.5 grams of the powdered material that had been dried was boiled in 20 milliliters of water and then filtered. It was determined if the coloration was blue-black or brownish green-black by adding a few drops of 0.1% FeCl3.

Lead acetate test: Two milliliters of plant extract and two milliliters of distilled water were combined.

Shake well after adding 0.01g of lead acetate to the liquid. When precipitate and white turbidity appear, tannins are present.

Estimation of flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, and this resulted in the development of a yellow-orange hue.

H_2SO_4 test: A fraction of the extract was treated with Conc.H2SO4, and the creation of orange color was observed.

Estimation of carbohydrates

Molisch's test

To 2-3 ml of extract of each solvent, add a few drops of α-naphthol solution in alcohol, stir, and then add concentrate H2SO4 from the edges of the test tube. a ring of violet where two liquids meet.

Fehling's test

It is used to identify sugars that are declining. Solution A: Dissolve 34.66 grams of copper sulfate in 500 milliliters of purified water. To make up to 50 milliliters of distilled water, dissolve 50 grams of sodium hydroxide and 17.3 grams of potassium sodium tartrate (Solution B). Mix two solutions in the same volume before using. Boil Fehling's A and B solution in 1 mL of mixture for 1 minute. Add the test solution in equal portions. Put in a kettle of boiling water and heat for five to ten minutes. Brick red flashed, and was followed by yellow.

Estimation of terpenoids

Five milliliters of the aqueous plant extract and two milliliters of chloroform were combined, allowed to evaporate on the water route, and then heated to a boil with three milliliters of concentrated H2SO4. As terpenoids took shape, a grey coloring appeared.

Estimation of steroids

Two milliliters of chloroform and five milliliters of aqueous plant crude extract were mixed with concentrated H2SO4. The lower chloroform layer began to become red, indicating the presence of steroids.

Formulation of polyherbal formulation

Measure and weighed the lozenge raw material including all the fine powders of Aparajita, tulsi, mulethi and black pepper as given in the formulation table. Taken the given quantity of water and excipients in vessel, it and boiled the mixture for 15 minutes. After boiling, cool the mixture and filter it. The filtrate was again taken in a vessel and premix of lemon, sugar and honey was added to it. This mixture heated until the desired consistency was obtained. Once desire consistency is obtained the preparation was poured in a silicon moulds. Cool lozenges for 20-30 minutes in room temperature before storing into refrigerator to form a hard formulation, after proper drying, the lozenges were removed from the moulds. The preparation was stored in glass container coated with fine sugar, at room temperature, away from moisture (Pundir S, Varma, 2014; Dahiya et al. 2015).

Table 1. Formulation table

Ingredients	Quantity (g)
Clitoria ternatea	4.0
Glycyrrhiza glabra	3.0
Ocimum sanctum	4.0
Piper nigrum	3.0
Lemon	q. s.
Sugar	q. s.
Honey	q. s.
Water	q. s.

Evaluation parameters (Kirti et al., 2015; Taubenberger and Palese, 2006)

Organoleptic properties

The formulation developed in the laboratory were evaluated for its acceptance based on visual observation for various organoleptic properties like Colour, Odour, Taste, Texture, Shape.

Uniformity of weight

Twenty formulations were weighed using a digital balance and the test was performed according to the official method. Ten lozenges were randomly selected from each batch and individually weighed. To The average weight and standard deviation of 20 lozenges were calculated. The batch passes the test for weight variation test if not more than 2 of the individuals lozenges weight deviates from the average weight. Yielding value between 90- 110% of average weight.

Calculation was done by using the following formula.

Average weight = Weight of 20 Lozenges /20 Weight variation

Uniformity of weight= Individual Wright- Average Weight x 100% /Average Weight

Disintegration time studies

Disintegration time is the interval required for complete disappearance of a lozenges or its particles from the tester. Test of the prepared lozenges was performed according to USP30. By using a disintegration tester through the disintegration medium of phosphate buffer with pH 6.2 maintained at 37 ± 0.5°C. The lozenge of optimized batch disintegrated in 90Seconds which is acceptable for throat Lozenges. Disintegration time was also within acceptance criteria of 90 seconds to 1.5 minutes depending up on type of lozenges.

Friability

The friability of formulations was determined using Roche Friabilator. It is expressed in percentage (%). Ten formulations were initially weighed and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes. The formulations were weighed again after taking out tables and brushing the dust away. If formulations are found broken or cracked and the final value exceed the limit test is consider failed. The value should be no more than 1% (0.5-1.0%). If exceed repeat three time for overall estimation. The % friability is then calculated with help of following formula:

Friability= (Initial Weight -Final Weight) X 100/ Initial Weight Friability

Measurement of pH

The acidity or alkalinity of lozenges was indicated by using lab pH meter, a scale from 1.0 to 14.0. 1% w/v solution was prepared by dissolving 1 g candy in 100 ml distilled water and its pH was recorded.

Determination of moisture

This test is used to determine the water content of a material by drying a sample to constant mass at a specified temperature. By the gravimetric method, 1 g sample was weighed and placed in an oven at 100-120°C for 3hrs. Cool to room temperature. Repeat until constant weight observed. Percentage friability is given by the equation.

% F = (Initial Weight-Final weight/Initial weight) ×100

Determination of Thickness

The thickness of the formulations was determined by using vernier calliper. Five formulations were used. The average values were calculated.

Average thickness = Total 5 lozenges thickness ×100/5

Hardness

Hardness indicates the ability of a formulation to withstand mechanical shocks while handling. The hardness of the formulations was determined using Monsanto hardness tester. It is expressed in kg/cm2. Three formulations were randomly picked and hardness of the formulations was determined.

Diameter

Ten formulations for diameter uniformity are carried out. Then the value of the diameter is taken. The deviation of each is calculated and the deviation of individual unit from the mean diameter should not exceed ± 5% for tablets with diameter of less than 12.5 and ± 3% for diameter of 12.55mm or more.

Pharmacological activity

Antimicrobial activity

The antimicrobial activity of the polyherbal formulation was assessed using the agar well diffusion technique. Plates of Mueller Hinton agar was prepared and sterilized by placing them in an autoclave set at 121°C for 15 to 20 min. The next step was to pour the sterile media onto the previously sterilized Petri plates and then to wait for them to cool to room temperature. The bacterial suspension containing *Streptococcus mutans* and *Staphylococcus aureus* was uniformly distributed on the agar plates using sterile cotton swabs. Using a sterile polystyrene point, we formed wells with a diameter of 9 mm in the agar plates. The wells were subsequently filled with polyherbal formulation at varying concentrations (25 µg, 50 µg, 100 µg). The plates were placed in an incubator set at a temperature of 37°C for 24 hr. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in mm and the value was used to compute the zone of inhibition (Eswar et al. 2016).

Anti-inflammatory

Bovine serum albumin denaturation assay

About 0.05ml of polyherbal formulation at various doses (10-50 g/ml) was combined with 0.45ml of bovine serum albumin. The pH was adjusted to 6.3. Following a 10 min interval at room temperature, it was incubated in a water bath that was heated to 55°C for 30 min. The standard group utilised was diclofenac sodium, while the control group used was dimethyl sulphoxide. The samples were then spectrophotometrically analysed at 660 nm. The protein denaturation percentage was calculated (Adetutu and Olukorede, 2021).

Egg Albumin denaturation assay

The egg albumin denaturation experiment was conducted using 2.8ml of phosphate buffer and 0.2ml of fresh egg albumin. Various amounts of polyherbal formulation (10-50g/ml) were added to the reaction mixture. We adjusted the pH to 6.3. After 10 min at room temperature, it was incubated in a water bath at 55°C for 30 min. The standard group utilized was diclofenac sodium, while the control group used was dimethyl sulphoxide. The samples were then spectrophotometrically analysed at 660nm. The protein denaturation percentage was calculated (Kumari et al. 2015).

RESULTS AND DISCUSSION

Percentage yield

Table 2. Percentage yield

Plant	Theoretical	Practical	% Yield
Clitoria ternatea	100	23.45	23.45
Piper nigrum	100	18.26	18.26
Glycyrrhiza glabra	100	16.32	16.32
Ocimum sanctum	100	22.40	22.40

5.2 Preliminary phytochemical estimation

Table 3. Preliminary phytochemical estimation

Phytochemical test	*Clitoria ternatea*	*Piper nigrum*	*Glycyrrhiza glabra*	*Ocimum sanctum*
Alkaloids	+	++	+	++
Phlobatannins	++	_	_	_
Glycosides	+	+	++	+
Tannins	++	++	+	+
Saponins	+	_	+	++
Terpenoids	+	_	_	+

Steroids	+	_	+	++
Flavonoids	++	++	++	++
Phenols	+	++	+	++
Reducing sugars	_	_	+	_
Proteins	+	+	+	+
Carbohydrate	+	_	++	+

Evaluation parameters

Organoleptic characteristics

Polyherbal lozenges were observed as rough texture, square in shape, sweet in taste, pleasant odor and greenish-brown on color.

Table 4. Organoleptic characteristics of polyherbal lozenges

Parameter	Observation
Color	Greenish-brown
Odor	Pleasant
Taste	Sweet
Shape	Square
Texture	Rough

Uniformity of weight

Table 5. Uniformity of weight of polyherbal lozenges

Formulation	Uniformity of weight (g)
Polyherbal lozenges	25.6

Disintegration time

Table 6. Disintegration time of polyherbal lozenges

Formulation	Disintegration time (min)
Polyherbal lozenges	4.0

Friability

Table 7. Friability of polyherbal lozenges

Formulation	Friability (%)
Polyherbal lozenges	0.69

Measurement of pH

Table 8. pH of polyherbal lozenges

Formulation	pH
Polyherbal lozenges	7.2

Determination of moisture

Table 9. Moisture content of polyherbal lozenges

Formulation	Moisture content (%)
Polyherbal lozenges	39.0

Determination of Thickness

Table 10. Thickness of polyherbal lozenges

Formulation	Thickness (mm)
Polyherbal lozenges	1.9

Determination of Hardness

Table 11. Hardness of polyherbal lozenges

Formulation	Hardness (kg/cm^2)
Polyherbal lozenges	6.0

Diameter

Table 12. Diameter of polyherbal lozenges

Formulation	Diameter (mm)
Polyherbal lozenges	2.0

Pharmacological activity

Evaluation of anti-microbial activity

Agar well diffusion method

Table 5.13 Anti-microbial activity of Polyherbal lozenges

Treatment	Zone of inhibition (mm)	
	S. mutans	*S. aureus*
Polyherbal lozenges	4.72	5.29
Gentamycin	9.0	11.0

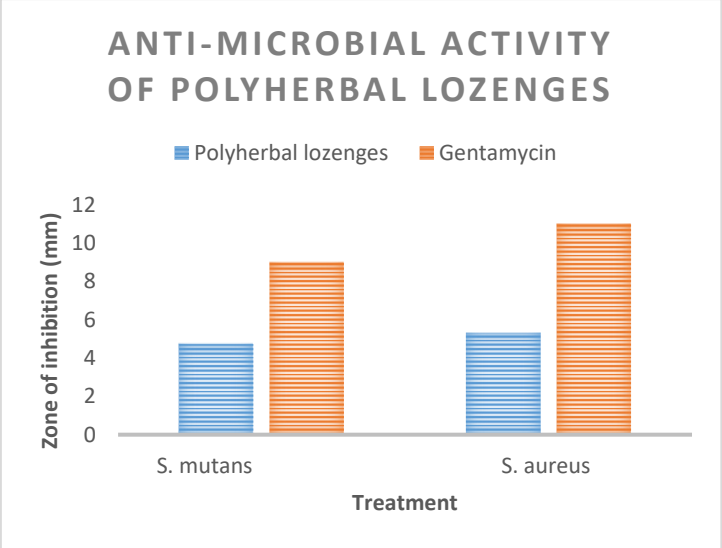


Fig 5.1 Anti-microbial activity of Polyherbal lozenges

Anti-inflammatory activity

Table 5.14 Bovine serum albumin denaturation assay of polyherbal lozenges

Conc. (µg/ml)	Percent Inhibition (%)	
	Polyherbal lozenges	Diclofenac sodium
50	51.27±2.34	69.28±0.35
40	43.68±3.22	57.19±0.62
30	39.53±2.14	48.23±0.56
20	34.20±1.49	43.12±0.71
10	27.34±4.29	24.57±0.18

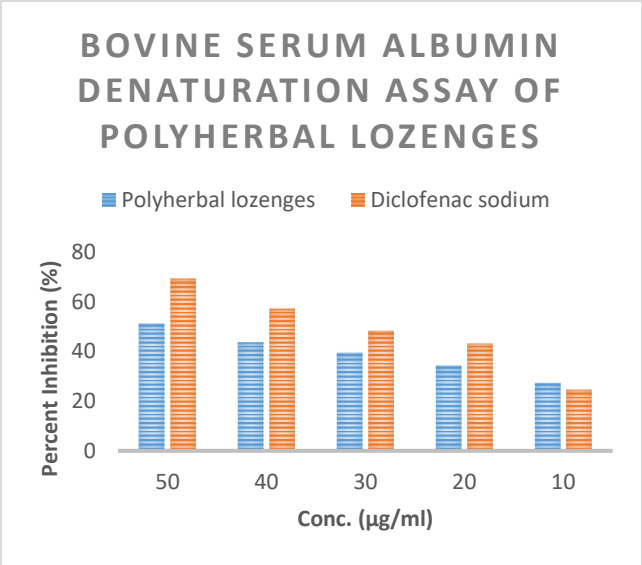


Fig 5.2 Bovine serum albumin denaturation assay of polyherbal lozenges

Egg Albumin denaturation assay of polyherbal lozenges

Table 5.15 Egg Albumin denaturation assay of polyherbal lozenges

Conc. (µg/ml)	Percent Inhibition (%)	
	Polyherbal lozenges	Diclofenac sodium
50	49.28±1.52	67.32±0.09
40	42.16±2.40	55.11±0.47
30	37.21±1.29	47.16±0.36
20	31.28±2.36	40.30±0.51
10	23.10±1.24	21.43±0.14

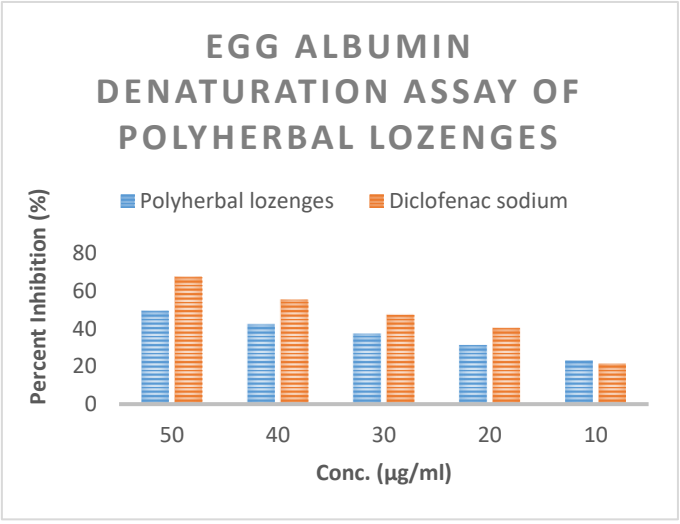


Fig 5.3 Egg Albumin denaturation assay of polyherbal lozenges

Modern research has focused on plant-based drugs used in the traditional treatment of illnesses, including inflammation, since they are low cost, have great therapeutic potential, and have few of the negative effects of synthetic medicines. The present study evaluated the anti-inflammatory qualities of S. aethiopicum's aqueous extract and showed how effectively it decreased inflammation in in vitro inflammatory models. Pharmaceutical companies are particularly interested in analyzing the phytochemical components of plants to screen their biological activity and create new drugs (Okwu and Uchenna, 2009). Other publications reported that C. ternatea leaves had antimicrobial compounds. One study looked at the active chemical components and phytochemical traits of C. ternatea. Understanding the therapeutic dynamics of medicinal plants requires biological screening and phytochemical analysis. Therefore, it has been demonstrated that C. ternatea has significant phytoconstituents in total phenols (245.14 ± 6.97 mg TAE · g−1), which is much higher than tannins (78.75 ± 2.09 mg TAE · g−1), followed by flavonoids (0.48 ± 0.96 mg RE · g−1). C. ternatea protects plants from environmental stress and alters human biological responses in addition to being a source of medicinal agents. Since it can shield Pisum sativum seed from Fusarium oxysporium, the ethanolic leaf extract of C. ternatea has also been investigated for its antifungal qualities. This indicates that C. ternatea can be utilized as an antifungal agent in medicine. Additionally, C. ternatea leaf ethanol extract may be used to treat candidiasis and cryptococcosis. All medicinal plants with high antiviral activity, including C. ternatea, have been categorized in order to study antiviral activity in Southern India. This tropical Asian flower showed exceptional antiviral efficacy when tested with anti-coronavirus extracts using the virucidal protocol (Pandey et al. 2013). The production or release of mediators at the site of injury is one feature of carrageenan-induced oedema. It is thought to be biphasic, with serotonin and histamine being created during the first hour and prostaglandins and cyclooxygenase (COX) products driving the second phase, which lasts longer than an hour. Kinins offer continuity between the two eras. 100 mg/kg of O. sanctum aqueous leaf extract significantly reduced inflammation (13.43% inhibition at 2 hours) ($p<0.001$) in our study. further shown the important anti-inflammatory effects of O. sanctum leaf essential oil extract (Eugenol) at a 33% inhibition level of 100 mg/kg. Amino acids like creatine and isoleucine may be responsible for O. sanctum's anti-inflammatory properties. According to Singh's research, O. sanctum contains linoleic acid, which may be the cause of its anti-inflammatory properties by blocking the lipoxygenase and COX pathways of arachidonate metabolism (Singh, 1998). We found that piperine significantly decreased the production of two important proinflammatory mediators, PGE2 and IL6, in IL1β-stimulated human FLS. This result was consistent with other studies that shown potent anti-inflammatory effects in several systems. Restricting the production of PGE2 is essential since it is a key contributor to pain. Additionally, MMP1 and MMP13 collagenases are important in RA and osteoarthritis because they are the rate-limiting components of the collagen breakdown process. The significant reduction in MMP13 synthesis is particularly significant as MMP13 is highly active against collagen type II, the primary collagen in cartilage, and degrades a range of collagenous and non-collagenous extracellular matrix macromolecules. To the best of our knowledge, this is the first report showing that piperine decreased MMP13 expression in IL1β-activated FLSs. We also investigated the molecular mechanisms underlying piperine inhibition. We found that piperine did not significantly reduce the activation of the MAP kinase or IκB kinase signaling pathways. At the highest dosage tested (100 μg/ml), piperine reduced the phosphorylation of ERK1/2 caused by IL1β. Piperine also prevented the transcription factor AP-1 from being activated in our system, but not NFκB. However, piperine may inhibit the activation of several transcription factors, including NFκB, c-FOS, cAMP response element binding, and activating transcription factor 2 (ATF-2), according to a previous study utilizing B16F-10 melanoma cells. As a result, it significantly reduced the production of TNF-α, IL6, IL1β, and granulocyte-macrophage colony stimulating factor (GM-CSF) in that study (Pradeep and Kuttan, 2004).

CONCLUSION

In conclusion, polyherbal lozenges of *Clitoria ternatea* (leaves), Black pepper (seeds), *Glycyrrhiza glabra* (root), and Tulsi (leaves) exhibited the

antimicrobial potential when tested against the *S. mutans* and *S. aureus* microbial strains. It also demonstrated significant anti-inflammatory activity through protein denaturation. Therefore, the polyherbal lozenges of *Clitoria ternatea* (leaves), Black pepper (seeds), *Glycyrrhiza glabra* (root), and Tulsi (leaves) can be utilized in the management of pain and inflammation.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

FUNDING

Nil.

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HOW TO CITE: Akash Kumar Tripathi*, Tarkeshwar Dubey, Development and Evaluation of a Polyherbal Formulation for the Treatment of Sore Throat, J. Pharm. Sci., 2026, 2 (6), 495-506. https://doi.org/10.5281/zenodo.20775527