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Research Article

Development and Evaluation of Polyherbal Mouth Ulcer Gel

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ABSTRACT

Mouth ulcers are painful lesions affecting the mucosal lining of the oral cavity and commonly cause discomfort during eating, drinking, and oral hygiene practices. Although usually self-limiting, effective treatment is required to alleviate pain and accelerate healing. Topical gel formulations provide rapid and localized therapeutic action while avoiding first-pass metabolism, making them suitable for managing oral lesions. The present study aimed to formulate and evaluate a polyherbal mouth ulcer gel containing extracts of Tulsi, Neem, and Liquorice. The herbal extracts were prepared by the maceration method using ethanol as the solvent. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, glycosides, tannins, and carbohydrates, which are known for their therapeutic and antioxidant properties. The gel was formulated using Carbopol 980 as a gelling agent along with PEG 400, honey, triethanolamine, methyl paraben, and propyl paraben through a simple mixing method. The prepared formulation was evaluated for various physicochemical parameters, and the results were found to be within acceptable limits. The developed polyherbal gel exhibited significant antioxidant activity, suggesting its potential as a safe, effective, and promising herbal remedy for the management of mouth ulcers.

INTRODUCTION

Mouth ulcer is an ulcer that occurs on the mucous membrane of oral cavity. Ulcers are consistently occur in the oral region. The ulcer shows symptoms such as redness, warm sensation & pain.

Although mouth ulcers can be miserable, particularly when you eat, drink or brush your teeth, they are generally harmless most of the time mouth ulcers will clear up by themselves within a week or two. Need to look dentist if the ulcer gets worse or lasts for longer than three weeks, or if you develop ulcer regularly.

Mouth ulcers are painful round or oval ulcers that form in mouth, especially inside cheeks or lips. A mouth ulcer is fracture or fracture of mucous membrane, located in the middle of the mouth. It

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is usually yellow or white and usually looks like pressure on the mouth which is mucous membrane.[1] One of the most prevalent pathologic disorders involving ulcers in oral mucosal membrane is Recurrent Aphthous Stomatitis [RAS]. Recurrent Aphthous Stomatitis causes single or multiple chronic ulcer in one oral mucosa, which is painful.[2]

Causes of Mouth ulcer

- Foods high in acidity or spice.
- Burns from chemicals that are preset in toothpaste or oral rinses.
- Medication including beta-blockers & pain killers.
- Anxiety or stress.
- Genetic factors.[2]

Types of mouth ulcer

Based on the size of lesions and number.

- Minor ulcers: - These are about 2-8mm wide in usually rot in 10 days to 2 Weeks
- Major ulcers: - Big in Size & depth are larger deeper than those are that are small. It can take up to 6 weeks to repair these uneven edges.
- Herpetiform wounds: - This type of wound is group of small wounds that have size of a pinhead.[1]

Treatment of mouth ulcers may include antiseptic mouth washer, antibiotic or antimicrobial gel formulations. Topical gels intended for the application on skin or to certain mucosal surfaces for local action or percutaneous penetration of medicament preparations.

A gel is a solid or semisolid preparation made up of at least two components that contains a condensed mass & is interpenetrated by liquid. Gels are made up of tiny quantity of solids scattered in big amount of liquid, however they have a solid like rather than a liquid like consistency.[2]

Herbal gel is a topical product formulated with natural plant extracts & ingredients. It often contains blend of herbs, essential oils & other plants-based compounds known for their beneficial properties, therefore are widely used due to their efficiency & lesser side effect

Advantages of gel: -

- Adesion: Gels can adhere better to the ulcerated area providing more consistent contact & relief.
- Soothing & Healing Properties: Gels often contains ingredients with healing properties which can help to reduce pain & discomfort associated with mouthulcer.
- Targeted Application: Gels can be applied directly to the ulcer, providing localized relief & reducing the systemic effects compared to the local medications.
- Comfort: Gels can be more comfortable & less obstructive than patches / films.
- It avoids first pass metabolism. [2]

Our primary objective is to develop a polyherbal gel for treatment of mouthulcer, therefore we are processed with three herbal natural ingredients such as Tulsi, Neem & Liquorice.

Tulsi :

The Tulsi plant is one of the most valued holistic medicinal plants in traditional India. Its herbal derivatives have been used as a household remedy for several aliments since time immemorial. It has been used to prepare several Ayurvedic herbal properties namely anti- oxidative, anti-microbial, anti-stress, anti-diabetic, anti-viral and many others that's why this plant is also given the term "Queen of herbs".[3] Tulsi extract shows inhibitory

effects against pathogen such as Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Proteus Mirabilis, Salmonella typhimurium. The Tulsi leaves extract has some quantity of volatile oil which contain phytochemicals such as aldehyde, terpenes (sesquiterpenes, monoterpenes) and phenols and it also contains some quantity of saponins, taninns, glycosides, quinone, phlobatanin, flavonoids (orientin and vicenin), steroids, coumarin and alkaloids. Tulsi is used in medicines and has various therapeutic properties and many useful phytochemicals which act as antimicrobial agents against pathogenic microbes.[4]

Neem :

Neem is a tree in the mahogany family Meliacea. The Neem extractsare found to be antimicrobial, antifungal, antiviral, antibacterial and antidiabetic. The chemical constituents and phytoconstituents of Neem are biologically active. Compounds may include secondary metabolites like flavonoids, steroids, tannins, terpenoids, saponins in varying concentrations. Azadirachtin, terpenoid is a low toxic compounds. An antimicrobial is an agent, which kills or inhibit the growth of microorganisms. Neem shows antimicrobial activity against some microorganisms. [5]

Liquorice

Liquorice is a perennial herb, belonging to family fabaceae. The liquorice extract is found to be antimalarial activity, anti- inflammatory, anti-fungal, antiulcer, anti-bacterial, anti-viral, anti-allergic. Chemical constituents & phytoconstituents of liquorice are flavonoids, isoflavones, saponins, proteins, alkaloids, coumarins, Glycirhizine is main constituents. Glycyrrhizic acid includes invitro activity, which works against H-pylori. It also acts as good Antioxidant agent. It is advantageous for increasing obstruction of gastric & upper respiratory tract & ulcer too.[6]

AIM AND OBJECTIVES

Aim: The main aim was to develop and evaluate the Polyherbal mouth ulcer gel

Objectives:

1. **Pre formulation studies**.

- To collect fresh leaves of Tulsi, Neem and dried roots of Liquorice.
- To obtain leaves and root extract using alcohol by cold maceration method.
- Phytochemical screening of extract.

2. **Post formulation studies**.

- Preparation of mouth ulcer gel.
- Evaluation of mouth ulcer gel.

MATERIALS AND METHODOLOGY

Preformulation Studies

Extraction of plant material by using cold maceration method:

A. TULSI: [7]

- Green and fresh Tulsi was collected from the plant.
- The leaves were cleaned by distilled water and then leaves were separated from the branches manually.
- The leaves were allowed for air drying under room temperature.
- The dried leaves were blended to a powder by grinder and stored in air tight glass container until required for preparation.

- The 50gm of crushed raw material was subjected to maceration with 200ml of absolute ethanol in beaker and sealed with aluminium foil and kept in the dark for 7 days.
- The beaker was stirred to ensure uniform and complete extraction.
- The mixture was filtered by using clean muslin cloth and the filtrate was collected in a cleansed beaker.
- The filtrate is concentrated using rotary evaporator and then air dried.

MACERATION OF TULSI

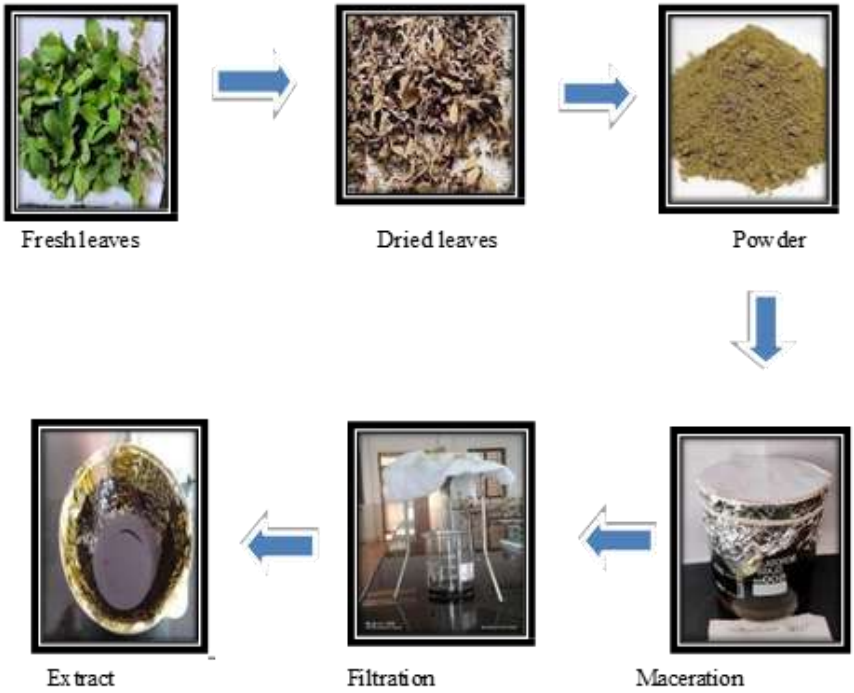

FIGURE 1:MACERATION OF TULSI

B. NEEM [8]

- The fresh leaves were collected, and washed in a tap water, rinsed ina sterile distilled water and dried for 10 days under room temperature.
- The dried leaves were then blended to powder a clean grinder and stored in an air tight glass containers until required for preparation.
- The 50 gmof leaves of Neem were weighted into beaker and 200 ml of ethanol were added and left to extract at room temperature.
- The mixture was filtered using a clean muslin cloth, and the filtrate was collected in a cleansed beaker.
- The filtrate was then concentrated with a rotary evaporator and subsequently air-dried.

MACERATION OF NEEM

FIGURE 2: MACERATION OF NEEM

C. LIQOURICE[9]

- Dried roots of Liquorice were collected.
- The dried roots were then blended to powder with clean grinder and stored in air container until required for preparation.
- 50gofpowder of Liquorice were weighed into beaker and200ml ofwas added and left to extract at roomtemperature.
- The beaker was stirred to ensure uniform and complete extraction.
- The mixture was filtered by using clean muslin cloth and the filtrate was collected in cleansed beaker.
- The filtrate is concentrated using rotatory evaporator and then air dried.

MACERATION OF LIQUORICE

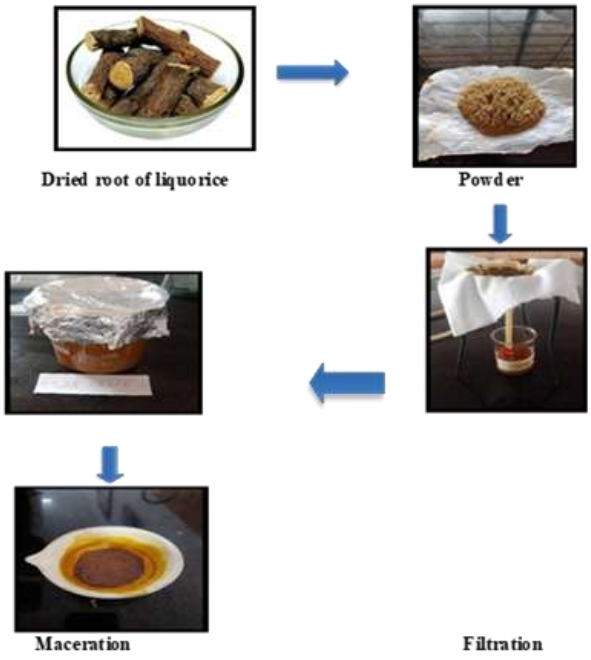

FIGURE 3: MACERATION OF LIQUORICE

Preliminary Phytochemical Screening [10]

Test for alkaloids:

1. Dragendroff's test: To 2-3 ml filtrate add few drops of Dragendroff's reagent. Orange brown precipitate is formed.

2. Wagner's test: 2-3ml filtrate with few drops of Wagner's reagent gives reddish brown precipitate.

Test for carbohydrates:

1. Molisch test: 2-3ml aqueous extract add few drops of alpha-naphthol solution in alcohol shake and conc. H2SO4 from the sides of the test tube violet ring is formed at the junction of two liquids.

2. Fehling'stest: Mix1 ml Fehling's A and1ml Fehling's B solutions, boil for1min. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow, then brick red is observed.

Test for flavonoids:

1. Shinoda test: To dry powder or extract, add 5 ml. 95% ethanol, few drops conc.HCl and 0.5gm magnesium turnings.Pink color observed.

2. Lead acetate test: To small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed

Test for glycoside:

1. **Killer-killani test:** To 2ml extract, add glacial acetic acid, add 1 drop of 5 % FeCl3 and conc.H2SO4 reddish brown colour appears at the junction of two liquid layers and upper layer appears bluish green.

2. **Foam test:** Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

Test for tannins:

1. **Gelatin test:** To 2-3 ml aqueous or alcoholic extract, add few drops of gelatin reagent white precipitate observed.

2. **5% FeCl3 solution:** To 2-3 ml of aqueous or alcoholic extract, add few drops of 5% FeCl3 solution deep blue-black color observed.

Test for proteins:

1. **Millons test:** Mix 3ml test solution, add 5ml millons reagent. White ppt, warm ppt gives brick red colour or ppt dissolves gives red colour solution.

2. **Precipitation test :**In sample add5% lead acetate,white colloidal ppt.

FORMULATION OF MOUTH ULCER GEL

Table 1: Formulation of mouth ulcer gel

Sr. No.	Ingredients	F1	F2	Category
1	Tulsi	1gm	1gm	Active Ingredients
2	Neem	1gm	1gm	Active Ingredients
3	Liquorice	1gm	1gm	Active Ingredients
4	Carbopol980	0.5gm	1gm	Gelling Agent
5	PEG400	5ml	5ml	Co-solvent
6	Methyl Paraben	0.03gm	0.03gm	Preservative
7	Propyl Paraben	0.03gm	0.03gm	Preservative

8	Triethanolamine	Q.S	Q.S	Adjust pH
9	Honey	0.5ml	0.5ml	Sweetening Agent
10	Distilled Water	Q.S	Q.S	Solvent
	Total	10gm	10gm	

PREPARATION OF GEL[2]

1. The herbal mouth ulcer gels were prepared by simple mixing method
2. Carbopol 980 kept for soaking overnight.
3. Take 5ml of water, add propyl &methyl paraben and heat it on waterbath.
4. After cooling add propylene glycol & mix all
5. Add honey, mix all ingredients & add Carbopol 980.
6. Triethanolamine added dropwise to adjust pH at last.
7. Make up the volume with distilled water.

Herbal extract
FIGURE 4: MOUTH ULCER GEL

FLOW CHART FOR PREPARATION OF GEL

Dissolve Carbopol 980 in distilled water
↓
5ml water + methyl and propyl paraben heat on the water bath
↓
After cooling add PEG400
↓
Neem, Tulsi& Liquorice extract mix in above mixture
↓
Volume was made upto 20 ml with the distilled water
↓
Mix all ingredients & add Carbopol980
↓
Triethanolamine added dropwise

POST FORMULTION STUDIES:

Evaluation parameter

- **Appearance:-**

The prepared gels were tested for colour, odour & homogeneity.

- **Measurement of pH:-**

The pH of herbal gel formulation was determined by using digital pH meter. 1gm of gel was taken and dispersed in 10ml of distilled water and keep aside for two hours. The measurement of pH of formulation was carried out in three times and the average values are reported. pH of gel formulation was reported.[2]

- **Spreadability:-**

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel that is placed in between the slides under the direction of certain load. If the time taken for separation for

two slides is less than better the spreadability. Spreadability is calculated by using the formula.

$$S=M*L/T.^{[2]}$$

Where,

M=Weight tied to upper slide L= Length of glass slides T=Time taken to separate the slides

- **Viscosity:-**

Viscosity of gel was measured using LMDV100

Viscometer with spindle number1.

- **Extrudability:-**

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 100 g was placed over the slides, and then, the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: Excellent, >80% extrudability: Good, and >70% extrudability: Fair) (11)

$$\frac{\text{Amount of gel extruded from tube}}{\text{Total amount of gel in tube}} \times 100$$

- **Anti-oxidant activity:-**

Prepare standard solution and control in each experiment as follows. Take test tubes and label as blank, control and test.

Blank : 600 µl Tris HCL.

Control: 100µl Ethanol + 400µl Tris HCL + 500 µl DPPH solution.

Test: 100 µl sample + 400 µl Tris HCL+ 500 µl DPPH solution.

Mix all the tubes and keep in dark for 30min. Read the absorbance at 490 nm.

Calculation

As = sample O.D.

Ac=control O.D.

$$\text{Inhibition Ratio \%} = \frac{Ac-As}{Ac} \times 100$$

RESULT AND DISCUSSION

PREFORMULATION STUDIES:

Extraction: Tulsi, Neem, & Liquorice has a good ability to extract solvent in ethanol. Maceration process gives better yield in Tulsi, Neem, & Liquorice.

Phytochemical screening: Table 2 represents a phytochemical Screening of the ethanolic extract of Neem, Tulsi & Liquorice.

Table 2: Results of phytochemical screening of extracts drugs

Sr. No	Phytoconstituents	Nameoftest	Tulsi	Neem	Liquorice
1	Alkaloids	Dragendroff's reagent	+	+	+
		Wagner's reagent	+	+	+
2	Carbohydrates	Molischtest	+	+	-
		Fehling'stest	+	+	-
3	Flavonoids	Shinodatest	+	+	+
		Leadacetate test	+	+	+
4	Glycosides	Killer-killani test	+	+	+
		Foamtest	+	+	+

5	Tannins	Gellatintest	+	+	-
		5%FeCl3	+	+	-
6	Proteins	Millonstest	-	-	+
		Precipitate test	-	-	+

PHYTOCHEMICAL SCREENING OF TULSI

FIGURE 05: PHYTOCHEMICAL SCREENING OF TULSI

PHYTO CHEMICAL SCREENING OF NEEM

FIGURE 06 : PHYTOCHEMICAL SCREENING OF NEEM

PHYTOCHEMICAL SCREENING OF LIQUORICE

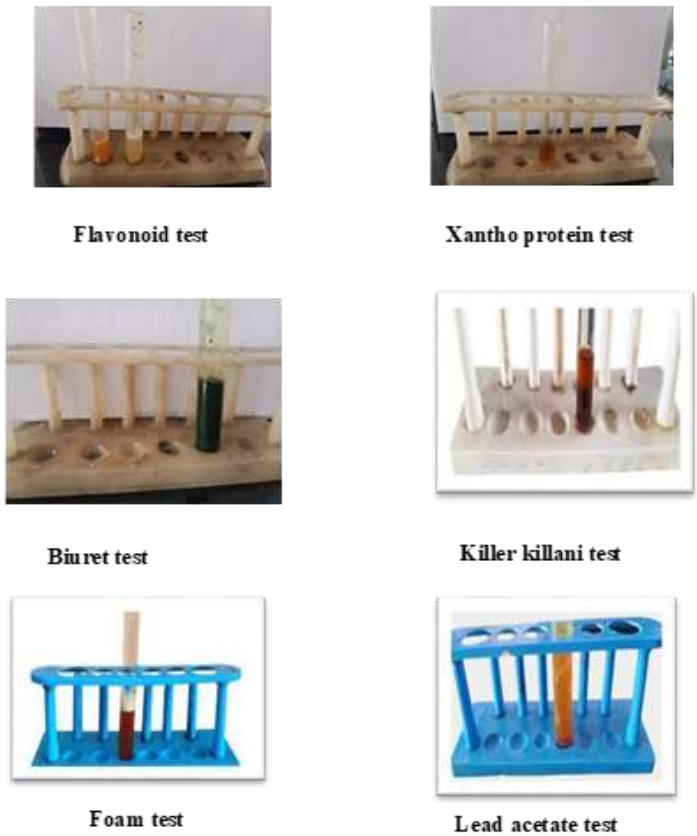

FIGURE 07 : PHYTOCHEMICAL SCREENING OF LIQUORICE

POST FORMULATION STUDIES

EVALUATION PARAMETERS:

Physical appearance:

Table 3:Result of Physical Appearance

Sr. No	Test	Result
1	Colour	Greenish
2	Odour	Characteristic
3	Homogeneity	Good

Measurement of pH:

Table 4: Result of Measurement of pH

Sr. No	Formulations	Result
1	F1	6.8
2	F2	6.7

Spreadability:

Table 5: Result of Spreadability

Sr. No	Formulations	Result (gcm/sec)
1	F1	21.87
2	F2	24.28

FIGURE 08: SPREADABILITY

Extrudability:

Table 6: Result of Extrudability

Sr. No	Formulations	Result(%)
1	F1	75%
2	F2	70%

FIGURE 09: EXTRUDABILITY

Viscosity:

Table 7: Result of Viscosity

Sr. No	Formulations	RPM	Result (mPascal)
1	F1	60	83.4
2	F2	70	75.8

FIGURE 10: VISCOSITY

Anti-oxidant activity result:

Table 8:Result of Anti-oxidant activity

Sr. No.	Sample	100%	50%	25%	12.5%
01	F1	13.7%	8.5%	3.3%	N.D

Note:

100% -1 mg in 1ml DMSO

Standard used - Vitamin c

SUMMARY

Polyherbal mouth ulcer gel containing natural ingredients for healing action was developed and evaluated using various parameters.

Tulsi, Neem & Liquorice was extracted using ethanol by cold maceration method.

These extracts were characterized by various chemical test like test for alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins etc.

Gel was prepared by using other ingredients such as Carbopol 980, PEG 400, Methyl paraben, Propyl paraben and Triethanolamine etc.

Developed formulation was evaluated for parameters like pH, Spreadability, Viscosity, Extrudability & Antioxidant activity.

The results obtained of the developed polyherbal mouth ulcer gel were found to be satisfactory.

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones.

Herbal formulation ha sgrowing demand in world market.

The study aimed to develop the herbal gel for mouth ulcer using extracts of Tulsi, Neem& Liquorice.

Desired formula of the gel was prepared & evaluated for their physicochemical properties like colour, odour, homogeneity, pH, spreadability, extrudability, viscosity & antioxidant activity.

From the studies it was concluded that prepared formulation showed good consistency, pH, spreadability, extrudability, viscosity and good antioxidant activity during study period of research which was effective.

From the above study it can be concluded that the polyherbal gel is safe to use as it was developed from herbal extracts & may be applied topically against mouth ulcer.

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