FORMULATION AND EVALUTION OF TOPICAL ANTIMICROBIAL AND ORAL ANTIULCER GEL FROM LIQUORICE EXTRACT

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ABSTRACT

This project is designed to develop licorice root into a herbal product and investigate its antimicrobial properties against various types of bacteria, including Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), and C. albicans, a species of yeast. Another property is antiulcer like mouth ulcer. It often causes pain and discomfort and may change the patient's food choices during treatment. The two most common types of mouth sores are localized sore throat and aphthous stomatitis. Canker sores are small, painful sores that usually have red edges and yellow-gray areas. Mouth ulcers can be treated with antibiotics, and corticosteroids, and there is also a way to treat mouth ulcers using herbs derived from the licorice plant.

Different extract concentrations. All prepared formulations have physical and chemical properties, choosing good appearance and consistency, permeability, recommended pH and physiological compatibility of the skin. The selected method was also characterized by examining the in vitro antimicrobial activity and antiulcer activity.

The results showed that licorice gel could be successfully made using Carbopol 934 as the gelling agent and the selected samples showed good antimicrobial activity against Staphylococcus aureus and Candida albicans, but not antibiotic against it. Pseudomonas aeruginosa. Therefore, the preparation of licorice topical gel can be considered a good alternative to antimicrobial in herbal medicine. The purpose of this study is to turn licorice into gel and to examine whether licorice is effective in the treatment of oral diseases.

Moreover, the results showed that the prepared licorice gel had good stability at room temperature and there was no significant difference after four months of storage ($p \leq 0.05$). A new herbal herbal antibiotic ointment gel formulation candidate has been proposed to enter the market.

KEY WORDS:- *Licorice, Antimicrobial, Antiulcer, CAR-934, HPMC, Mouth ulcer, Gel, Extract.*

1. Introduction:-

Gel formulations are homogeneous semi-solid formulations containing the drug or a dispersion of one or more drugs in a suitable hydrophilic or hydrophobic matrix, providing a suitable delivery system for the drug as they are not too oily and can be easily removed. skin.They are used for prevention, protection or healing on the skin or some mucosa. Licorice root is approved by the U.S. Food and Drug Administration (FDA) as a dietary supplement for many uses. [1]

The dried, peeled or unpeeled roots and stolons of Glycyrrhiza glabra Linn (Fabaceae family) are known as liquorice or licorice. For over 4,000 years, this plant has been utilized for its medical properties. Numerous conditions, including ulcers, arthritis, allergies, inflammation, leukemia, cancer, psoriasis, atopic dermatitis, and hepatotoxicity, have been successfully treated with licorice. Glycone and glycone, the two main ingredients of licorice, are what give it its therapeutic qualities. Glycone, also known as glycyrrhizic acid (GA), and aglycone, also known as glycyrrhetinic acid, are two different compounds. Of these, the glycone portion, or GA, is a significant one that gives licorice its pharmacological and biological characteristics. [2]

Triterpenoid GA is a diasaccharide glycoside that has been shown to have antimicrobial, anti-inflammatory, anti-diabetic, anti-allergic, and many other effects.Twenty triterpenoids and close to 300 flavonoids are among these compounds. Natural glycosides with significant structural diversity and bioactive properties are called triterpenoids.[3]

The need to create novel antimicrobial chemicals from other sources, such as medicinal plants, arose from the global growth in resistance to antimicrobial drugs. The introduction of herbal anti-microbials was made possible by the search for novel medications with stronger anti-microbial action and lower toxicity. Herbal antimicrobials can help down the expense of medication regimens while also helping to treat resistant illnesses. [5]

Topical gel's non-greasy texture, ease of administration, and ease of removal have led to its widespread adoption globally. There isn't a commercially available licorice gel in the Iraqi market, despite numerous studies on the use of licorice gel to treat dermatitis and aphthous ulcerations.Glycyrrhizic acid was extracted via maceration. The physical and phytochemical properties of licorice powder and roots are analyzed. The optimal licorice extraction solvent for glebridin and glycyrrhizic acid was selected. [18]

The extraction process used simple maceration. Orally administerable excipients are chosen for the gel's manufacture. Gel manufacture involves the use of excipients such as propylparaben, triethanolamine, methylparaben, propylene glycol, and carbopol 934. The gelling component carbopol 934 was used in three different concentrations to make the gel formulation. One batch is selected based on appearance, with the majority of the gel-like properties. [4]

1.1 PLANT PROFILE:-

Generally referred to as mulaithi, sweet wood, or liquorice, Glycyrrhiza glabra L. (Fabaceae) is a tiny perennial herb that is indigenous to central and southwest Asia as well as the Mediterranean region. Around the world, this plant is grown in China, Northern India, Italy, Russia, France, UK, USA, Germany, and Spain. It has given us access to several crucial bioactive components for medications that can save lives and are part of the arsenal of contemporary medicine. Nevertheless, only 6% of the estimated 250,000–400,000 plant species have been examined for biological activity, and 15% have been examined through phytochemical research.[1]

Fig. 1:- Glycyrrhiza glabra (liquorice) Plant

Fig. 2:- Dried Licorice Root And Licorice Powder

1.2 SCIENTIFIC CLASSIFICATION:-

- **Kingdom** - Plantae
- **Division** - Angiospermae
- **Class** - Dicotyledoneae
- **Order** - Rosales
- **Family** - Leguminosae
- **Genus** - Glycyrrhiza S
- **Pecies** - glabra Linn
- **Binomial Name** - Glycyrrhiza glabra L.

1.3 MORPHOLOGY:-

Glycyrrhiza glabra Linn is a shrub that rarely goes out of style that can grow as high as 2.5 meters. With four to seven pairs of oblong, elliptical, or lanceolate leaflets, the compound, imparipinnate, alternating leaves are present. The blooms are lavender to violet in color, narrow, usually papilionaceous, and borne in axillary spikes. The calyx is glandular hair bearing, short, campanulate, and has lanceolate tips. The fruit is an erect, glabrous, compressed legume or pod that can grow up to 1.5 cm in length. It has a few reticulate pits and often holds three to five brown reniform seeds. The taproot has three to five offshoot roots and is about 1.5 cm long.[6]

1.4 CULTIVATION AND COLLECTION: -

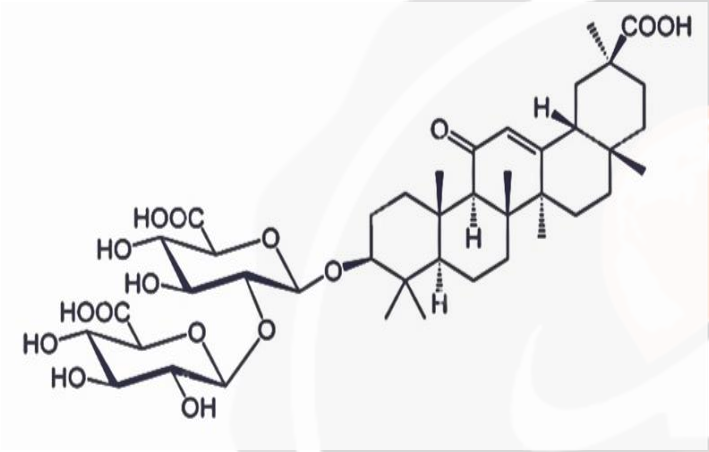
Spain and Italy are the two countries where Spanish licorice is grown extensively. The plant is multiplied by means of young stolons. They are divided into immature segments, with two to three aerial shoot buds on each segment. The plant requires deeply sandy, well-

prepared soil that has been amended with manure from the farm. The pieces of stolons are planted in March, two to three feet apart. Fertilizers ought to be applied when the green areas are developing. The crop is kept free of weeds.

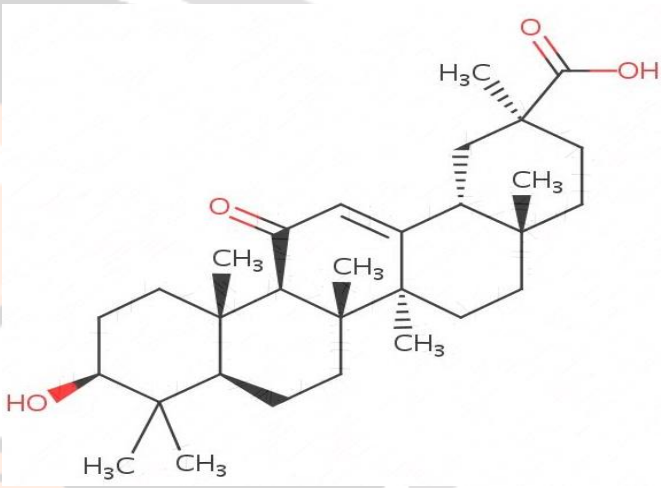
The roots are taken after three to four years, or when they have developed sufficiently. Rhizomes and roots are removed in October. especially from plants that are yet to bear fruit. Buds and rootlets are removed, and the medicine is cleansed. Some of the portions have been peeled and chopped into smaller bits. The drug loses around half of its weight during each of its two drying processes—one in the sun and one in the shade.[8]

1.5 PHYTOCHEMICAL CONSTITUENTS:-

Glycyrrhizin, a saponin glycoside found in the roots of Glycyrrhiza glabra Linn, is 60 times sweeter than cane sugar; Liquirtin, isoliquertin, liquiritigenin, and rhamnoliquirilin are among the flavonoid-rich fractions. Five novel flavonoids were also extracted from dried roots glucoliquiritin apioside, prenyllicoflavone A, shin flavanone, shinpterocarpin, and 1-methoxyphaseolin.



Glycyrrhizin



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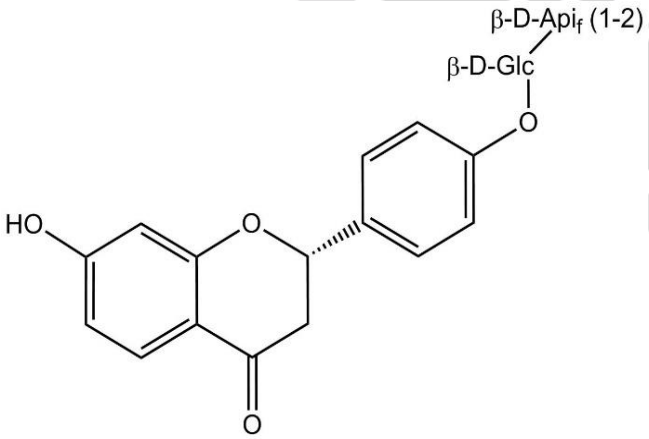
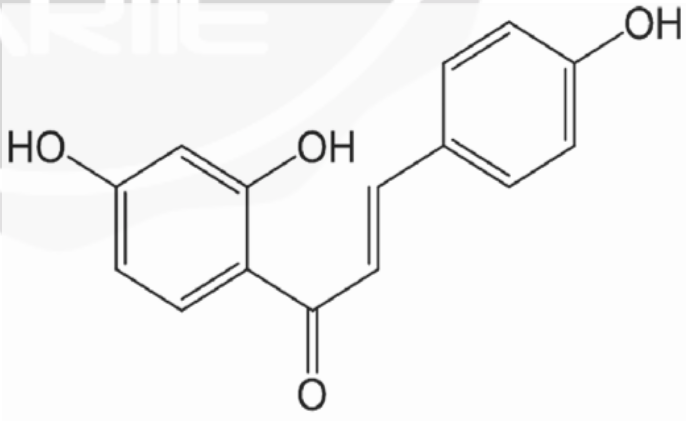
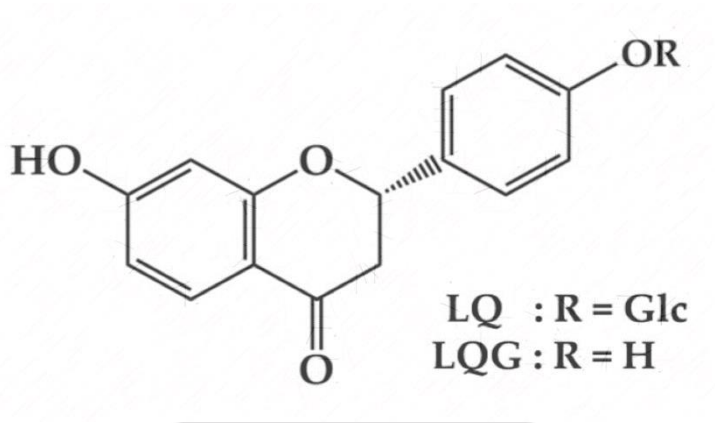


Isoliquiritinapioside



Isoliquiritigenin



Isoliquiritin and Liquiritigenin

Fig. 3:- Structure of Glycyrrhizin, Glycyrrhetinic Acid, Isoliquiritinapioside, Isoliquiritigenin, Isoliquiritin, Liquiritigenin.

Licopyranocoumarin, licoarylcoumarin, glisoflavone, and novel coumarin-GU-12 were also isolated, and their structures were determined. Isolated from roots are four novel isoprenoid-substituted phenolic constituents: licoriphenone, 1-methoxyficifolinol, isoangustone A, and semilicoisoflavone B.

Additionally, kanzonol R, a novel prenylated isoflavan derivative, was discovered. It has been reported that the roots contain a variety of volatile substances, including pentanol, hexanol, linalool oxides A and B, tetramethyl pyrazine, terpinen-4-ol, α-terpineol, geraniol, and others. The essential oil is also separated to determine the presence of propionic acid, benzoic acid, ethyl linoleate, methyl ethyl ketine, 2,3-butanediol, furfuraldehyde, furfurylformate, 1-methyl-2-formylpyrrole, trimethylpyrazie, maltol, and any additional chemicals. Several 2-methyliso-flavones and an uncommon coumarin, C liquocoumarin, 6 - acetyl-5 hydroxy-4 -methyl coumarin, are present in the Indian roots. There is also asparagine.

About 10–25% of licorice root extract is made up of glycyrrhizin, also known as glycyrrhizic acid or glycyrrhizinate, which is thought to be the main active component. Glycyrrhizin is a saponin molecule that consists of a disaccharide of glucuronic acid linked to a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid; enoxolone). The 18α and 18β stereoisomers of glycyrrhizin and glycyrrhetic acid are both possible. Glycyrrhizin, a tribasic acid, is found naturally in licorice root in the forms of potassium and calcium salts. It can create a wide range of salts. The Food Chemicals Codex has criteria for the ammoniated salt of glycyrrhizin, which is produced from licorice extracts and utilized as a food flavoring agent.An analogue of glycyrrhetic acid called carbenoxolone (18-β glycyrrhetinic acid hydrogen succinate) is used to cure ulcers.[16, 17]

1.6 PHARMACOLOGICAL PROPERTIES :-

- Antimicrobial activity
- Antiulcerative activity
- Antioxidant activity
- Anti-inflammatory activity
- Antitussic and expectorant activity
- Antiviral activity
- Hepatoprotective activity

- Neuroprotective activity
- Sedative activity
- Antidepressive activity
- Oestrogenic and androgenic effects

❖ ANTIMICROBIAL ACTIVITY:-

Microorganisms develop antibiotic resistance and adverse effects. Thus, there has been a lot of focus on finding solutions for physiologically active chemicals that have been identified from plant species and extracts. In place of antibacterial chemicals, medicinal herbs offer a natural supply. It has been known for a number of years that the antimicrobial activity of plant extracts and oils is related to saponins, alkaloids, flavonoids, glycosides, phenols, and tannin. Only a few research have demonstrated the efficacy of licorice roots against bacteria, despite previous studies describing the antimicrobial activity of roots and rhizomes.

The antibacterial activity of licorice root extracts in aqueous and ethanolic extracts was assessed. The antimicrobial activity of Klebsiella pneumoniae, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis was tested by measuring the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using the serial dilution method and the paper disc evaluation method.

The findings have verified that, depending on dosage, licorice's ethanolic extract possesses antibacterial potential against gram-positive bacteria and Candida albicans. Since licorice's ethanolic leaf extract is effective against gram-positive bacteria, it may be a viable substitute medicine for a variety of types. The antioxidant and antibacterial characteristics of methanolic root extracts of Glycyrrhiza glabra var. glandulifera were studied by Karahan F et al. Plant samples were gathered from the eastern Mediterranean region of Turkey. The disc-diffusion and MIC techniques were used to assess the antibacterial efficacy. Methanolic root extracts were found to be less effective than Gram-positive root extracts against Gram-negative bacteria in the results of the antimicrobial testing. Furthermore, compared to other bacteria, root methanolic extracts have demonstrated greater efficacy against Candida species. The study's findings indicate that the biological characteristics and chemical composition of common licorice vary depending on the environment. Furthermore, the study's findings supported licorice's historic uses and suggested that it would be useful in treating other infections.

When Glycyrrhiza glabra roots were investigated for their antibacterial impact, 500 µg/mL of antimicrobial potential was discovered. Glabridin, at a dosage of 29.16 µg/mL, has been shown through phytochemical research to potentially inhibit H (37) Rv strains and Mycobacterium tuberculosis (H37) Ra. It can therefore inhibit both Gram-positive and Gram-negative bacteria due to its antibacterial properties.[11]

❖ ANTIULCER MECHANISM :-

It is commonly recognized that G. glabra extract has antiulcerative properties. It is used to treat gastric and duodenal ulcers in the gastrointestinal tract (Bardhan, Cumberland, Dixon, & Holdsworth, 1978); nevertheless, it is also utilized as an adjuvant to treat spasmodic pain associated with chronic gastritis (Armanini et al., 2002). Since the 1970s, G. glabra has been used to treat duodenal and peptic ulcers; this traditional use is associated with the plant's anti-inflammatory saponins (Krausse et al., 2004). The primary substance in charge of this action is glycyrrhizin, which can increase the amount of prostaglandins in the digestive system and encourage the secretion of stomach mucus (Jafarian & Ghazvini, 2007).

Furthermore, licorice has an antipepsin action by extending the longevity of stomach surface cells (Ram, Lachake, Kaushik, & Shreedhara, 2010). Moreover, studies on deglycyrrhizinated liquorice in the treatment of gastrointestinal ulcers have revealed some effects, indicating the possibility of additional active components (Zadeh, Kor, & Goftar, 2013). In fact, it has been observed that carbenoxolone, an analogue of glycyrrhetinic acid, inhibits two key enzymes involved in prostaglandin metabolism: Δ13 prostaglandin

reductase and 15-hydroxyprostaglandin dehydrogenase. This increases prostaglandin levels and has been shown to improve gastric and duodenal ulcers in clinical trials (Damle, 2014). In actuality, prostaglandins promote the release of mucus and the proliferation of cells, which heal ulcers.

However, the usage of carbenoxolone, a derivative of glycyrrhetic acid, is limited due to its side effects, which include the possibility of developing pseudo aldosteronism. Bardhan et al. (1978) investigated the effects of oral liquorice treatment in 96 patients suffering from stomach ulcers. The deglycyrrhizinated liquorice or placebo treatments were given to the patients at random. But after four weeks, there were no variations in the percentage of ulcer area reduction or clinical enhancements between the groups. (1978, Bardhan et al.) [14]

❖ **Oral ulcers:-**

An ulcer is a molecular necrosis-induced rupture in the epithelium's integrity. Patients most frequently seek assistance from their physician or dental surgeon for mouth ulcers. Redness, burning sensations, and/or pain are typically the first complaints to manifest. They can appear anywhere in the oral cavity, but if they do so in a moveable place, they could hurt.

Fig.4:- Mouth Ulcer

A mouth ulcer, sometimes referred to as an oral ulcer or a mucosal ulcer, is an ulcer that forms on the mucous membrane of the oral cavity. These are usually painful round or oval mouth sores that appear on the inside of the cheeks or lips.Mouth ulcers are relatively common and can result from a number of illnesses and treatments, even though there are typically no significant underlying causes for them. Mouth ulcers are frequently caused by nutritional deficiencies such as iron deficiency, vitamin shortages (especially B12 and C), infections, stress, indigestion, mechanical injury, food allergies, hormonal imbalances, skin problems, etc. Aphthous ulcers, often known as mouth ulcers, mightache when you eat, drink, or clean your teeth. [21]

❖ **FACTORS RESPONSIBLE FOR THE MOUTH ULCERS:-**

- Toothpastes and mouthwashes that contain sodium lauryl sulfate
- Emotional stress / Psychic stress
- Hormonal changes
- Nutritional deficiencies

- Mechanical trauma
- Viral infections
- Allergies and sensitivities
- Genetics
- Infectious agents (both bacterial and viral)
- Medical conditions

2. MATERIALS AND CHEMICALS:-

2.1 Material:-

The dried coarse powder of licorice root was purchased from RESEARCH-LAB FINE CHEM INDUSTRIES Mumbai 400 002 (INDIA) A GMP certified company.

2.2 Chemicals:-

The suppliers of carbopol 934 (CAR-934), Hydroxy propyl methyl cellulose (HPMC) and triethanolamine were (RESEARCH-LAB FINE CHEM INDUSTRIES Mumbai 400 002 (INDIA) A GMP certified company); (MODERN INDUSTRIES STA, MIDC. Malegaon-Sinnar Dist. Nashik); (e-mail: sales@researchlab.in An 150 9001,14001& OHSAS 18001, GMP GLP certified company).

3. METHODOLOGY:-

3.1 PREPARATION OF EXTRACTS: -

Extraction is the process of removing the medicinally active ingredient from its parent source by means of appropriate standard techniques and selective solvents. Glycyrrhizin has been extracted from licorice using a variety of extraction techniques, such as analytical, solvent-based dipping, percolation, and maceration, microwave-assisted, Soxhlet, etc. The product yield was compared to other current techniques using a novel ultrasonic approach.

❖ **Dipping (Maceration) method :-**

The maceration or dipping method entails soaking coarse or powdered plant materials in a suitable solvent in a stoppered container and letting it stand at room temperature for a while. His method aims to rupture plant cell walls in order to liberate the targeted phytochemicals into the extraction solvent. The type of substance extracted from the samples is mostly determined by the solvent used, which is why it is the most important factor. For licorice powder, ethanol and water (30:70 v/v) were utilized as the solvent for 10g of licorice extract. To dissolve the glycyrrhizic acid in the extraction solvent, the root powder was immersed in it for a duration of seven to ten days.

After that, the liquid extract is stored in petri plates so that the solvent can evaporate and produce a dry liquorice extract. During the maceration process, the active chemical ingredients of crude pharmaceuticals are dissolved in a solvent. Glycyrrhizic acid and glabridin, the active ingredients in liquorice extraction, dissolve in ethanol:water (3:7). The solvent is evaporated using a rotary evaporator, and the dried active is then collected.[7, 16]

3.2 PHYTOCHEMICAL SCREENING: -

Using various assays and reagents, all of the aforementioned produced extracts were put through preliminary phytochemical screening tests to determine the presence of distinct components.

Test for flavonoids:- The extract was combined with sulfuric acid that had been concentrated. Anthocyanins are indicated by a yellow-orange color, while flavones are indicated by an orange to red colour.

Test for Saponins:- Take a small amount of each extract (Aq. and Alc.) and add it to 20 milliliters of distilled water. Shake the created solution lengthwise in a graduated cylinder for fifteen minutes after adding water. A 1cm layer of foam indicates the presence of saponins.

Test for terpenoids:- Trichloroacetic acid was applied to the extract as part of the terpenoids test (trichloroacetic acid test). The emergence of yellow color signifies the presence of terpenoids.

Fehling's test:- Extract was kept on the water bath to test for carbs. This was combined with Fehling solutions A and B. Reducing sugars were present as evidenced by the brick-red precipitate.[17]

3.3 FORMULATION OF GEL:-

- **PREPARATION OF LICORICE GEL USING CAR-934:-**

Various gel formulations were prepared using different concentrations of CAR-934 as the gelling polymer. These formulations are listed in Table 1; where CAR code is the code of formulations prepared with CAR-934. CAR-934 dispersion was prepared by adding 15 ml of distilled water to the polymer, gently mixing by hand, and then stirring at 100 °C using a magnetic stirrer (Fisher Scientific, Korea). Heat at C for 2-3 hours. Once cooled, cover the dispersion with a piece of aluminum foil and leave overnight to remove bubbles. Dissolve the preservatives (methylparaben and propylparaben) in propylene glycol using separate containers with magnetic stirrers (Fisher Scientific, 400 rpm, 25 -30 °C, 2-3 hours). The preservative is then mixed with the polymer dispersion using a magnetic stirrer (400 rpm, 2 hours) to obtain a clear dispersion. Mix licorice extract powder with a small amount of distilled water by hand for about 10 minutes until the drug level (extract solution) is formed, leave in the refrigerator. The next day, the extract was added to the mixture of antibiotic and polymer dispersion and mixed together (350 rpm, 2-3 rpm). As the last step to prepare the gel, add a few drops of triethanolamine and leave the gel recipe in the refrigerator.

Table 1. Composition of licorice gel, code CAR is presenting formulas prepared with CAR-934. Formula (CAR-3) is the selected formula.

Sr. No	Ingredients	Quantity			Role
		CAR-1	CAR-2	CAR-3	
1.	Carbopol 934	1%	1.5%	2%	Gelling agent
2.	Methyl paraben	0.02%	0.04%	0.04%	Preservative
3.	Propyl paraben	0.005%	0.005%	0.005%	Preservative
4.	Propylene glycol	10%	8%	10%	Base
5.	Extract	5%	5%	5%	Antiulcer activity
6.	Triethanolamine	s.q	s.q	s.q	pH adjustment
7.	Water	q.s	q.s	q.s	Aqueous base

❖ **PREPARATION OF LICORICE GEL USING HPMC:-**

Other formulations have been prepared using different HPMC as the gelling polymer; these are listed in Table 2; where code H gives the polymer formulation prepared here. HPMC dispersions were prepared by mixing one-third of the final volume of distilled water with the polymer in a beaker (350 rpm, 30 °C, 2–3 h). Food products
(methylparaben and propylparaben), packaged with two preservatives, dissolved in propylene glycol
mixed (400rpm, 25-30°C, 2 hours). Dissolve the dried licorice extract in a separate glass with a small amount of water as described to prepare the extraction solution. The next day, to prepare the final licorice gel prepration
add the contents of the three containers together, cover with a piece of aluminum foil and place in the refrigerator.

Table 2. Composition of licorice gel, code H is presenting formulas prepared with HPMC. Formula (H-4) is the selected formula.

Sr. No	Ingredients	Quantity				Role
		H-1	H-2	H-3	H-4	
1.	HPMC	2.5%	5%	10%	15%	Gelling agent
2.	Methyl paraben	0.02%	0.02%	0.04%	0.04%	Preservative
3.	Propyl paraben	0.005%	0.005%	0.008%	0.008%	Preservative
4.	Propylene glycol	10%	10%	12%	10%	Base
5.	Extract	5%	5%	5%	5%	Antiulcer activity
6.	Triethanolamine	s.q	s.q	s.q	s.q	pH adjustment
7.	Water	q.s	q.s	q.s	q.s	Aqueous base

4. EVALUATION OF LICORICE GEL:-

Visual Appeal and Uniformity Once the gel was poured into the container, the prepared formulations were visually inspected to ensure uniformity and appearance. Their hue, gel-like appearance, and aggregate presence have all been tested.

4.1 Calculating-PH:-

After precisely weighing a 0.5 g gel, it was diluted in 50 ml of purified water. After two hours, the pH of the dispersion was determined using a digital pH meter that had been calibrated at 4.0, 7.0, and 9.0 using standard buffer solution. Three separate pH readings were obtained, and average values were computed.

4.2 Skin irritation test:-

This test was run on each of the developed recipes to demonstrate the gel's skin compatibility. The purpose of applying it to human volunteers was to see if there was any irritation that would make using the gel difficult. A sample of five human volunteers was selected to assess skin irritancy. A surface area of over two square inches was treated topically with one gram of the tested gel. The five volunteers signed an informed consent form indicating their willingness to take part in the test. For approximately 24 hours, observations were made at regular intervals to look for any redness, lesions, irritation, edema, or other indication that the skin was irritated.

4.3 Spreadability Test:-

Good spreadability is a prerequisite for every topical application, but gel compositions in particular. The word "spreadability" refers to the amount of surface area that a solution will quickly spread when applied to skin or other afflicted areas. A homemade apparatus consisting of two glass slides with identical diameters was utilized to compute the spreadability of a formula. The spreadability was determined based on the gels' "slip" and "drag" properties. After applying too much gel—roughly 2 g—to the ground slide's surface and letting it move for a while, the spreadability was calculated as previously reported.15Preferable spreadability is indicated by a shorter time interval.

Spreadability (S) was determined using the formula:-

$$S = M.L/TW.$$

S stands for spreadability, M for mass attached to the upper slide (g), L for glass slide length (cm), and T for the amount of time the slide takes to move a certain distance (sec). [24]

4.4 Viscosity Determination:-

With spindle number 96 operating at 10 rpm, the viscosity of each created formulation was measured using the Brookefields viscometer LVDVE with helipath. Table displays the findings.

4.5 Extrudability:-

The gel formulations were filled in standard capped collapsible aluminium tubes and sealed to the end. The extrudability was determined by pressing of the thumb.[11]

4.6 Stability Study for the Selected Licorice Gel Formula:-

The chosen licorice gel composition was subjected to a 4-month stability test at 25±2°C while being stored in a tightly sealed container. Samples were examined at predetermined intervals of one, two, three, and four months. After four months, the chosen formula's physical characteristics were assessed, primarily looking at its color, consistency, spreadability, clog or aggregate presence, and appearance. The chosen formula was also assessed for additional factors, such as pH variation. [25]

4.7 In Vitro Anti-microbial Activity:-

Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans were the microorganisms employed in this investigation, and they were obtained from the clinical laboratory sciences department's microbiology laboratory at the D batu College of Pharmacy. Using

the disc agar diffusion method, licorice gel's antimicrobial activity was assessed. Following nutritional agar subculturing for the bacteria and Sabouraud dextrose agar subculturing for the yeast, the plates were incubated aerobically for 24 hours at 37˚C for the bacteria and 48–72 hours at 37˚C for the yeast.[14]

4.8 Anti-microbial Activity:-

❖ **Susceptibility Test:-**

A susceptibility test was conducted on one species of yeast (C. albicans) and two types of bacteria (S. aureus, a Gram positive bacteria, and P. aerogenosa, a Gram negative bacteria). To prepare the bacteria for the susceptibility test, four to five pure culture colonies of bacteria were mixed with three to five milliliters of Muller-Hinton broth. The bacterial suspension was then visually assessed to meet the 0.5 MacFarland criterion. After being immersed in bacterial inoculum suspension, a cotton swab was used to streak a 90 mm Muller-Hinton agar plate's whole dried surface. Conversely, a part of the C pure culture colony was mixed in with the yeast for the susceptibility test.

albicans into Sabouraud dextrose broth, and after that, the yeast suspension met the 0.5 MacFarland criterion qualitatively. After soaking a cotton swab in yeast inoculum suspension, the cotton swab was streaked over a 90 mm Sabouraud dextrose agar plate's whole dried surface.

❖ **Preparation of Discs:-**

Disks made of sterile filter paper Whatman No. 1 (6 mm radius) were saturated with various concentrations of 5% licorice gel made from stock concentration (5, 10, 15, 20, 25, 30 mg/ml) and left overnight for saturation. A stock concentration was made by dissolving 200 mg in 1 ml of distilled water.28 Using sterile forceps, the discs were placed on the inoculated Muller-Hinton and Sabouraud dextrose agar plates. The plates were subsequently inoculated for 24 hours at 37°C for bacteria and 24-48 hours for Candida albicans. In order to evaluate the antimicrobial activity, the diameter of the inhibitory zone was measured; the diameter was expressed in millimeters. Both types of bacteria were tested with 10µg of chloramphenicol, while Candida albicans was tested with 1µg of voriconazole as a control antibiotic.To guarantee dependability, the susceptibility test was administered three times, and the test's mean was computed. [12, 13 14]

5. RESULTS AND DISCUSSION:-

5.1 EVALUATION OF LICORICE GEL:-

The prepared gel has been prepared from licorice root extract. The extraction was carried out by simple method (hydroalcoholic extraction) and it was easy except the process of scratching from the petri dishes; however, the yield of extracting 100g in one liter 70% ethanol is 10 grams.

5.2 PHYTOCHEMICAL ANALYSIS:-

The tests performed for Phytochemical screening showed the presence of flavonoids, saponins, terpenoids and carbohydrates in the extract of Glycyrrhiza glabra.

Table. 3:- Phytochemical screening of glycyrrhiza glabra

Sr. No.	Phytoconstituents	Glycyrrhiza glabra
1.	Test for flavonoids	+
2.	Test for Saponins	+
3.	Test for terpenoids	+
4.	Test for carbohydrates	+

5.3 PHYSICAL EVALUATION OF GEL FORMULATION:-

Table. 4:- Physical evaluation of gel formulation.

Sr. No.	Parameters	Observations
1.	Colour	Yellowish green
2.	Consistency	Good
3.	Odour	Characteristics
4.	Gritty particles	No
5.	Appearance	Good
6	Extrudability	Good

5.4 SELECTION OF GEL WITH CONSIDERATIONS OF VISUAL APPEARANCE:-

Various formulations were created, accounting for the gelling agent concentration and the formula's primary constituent. The latter's concentration is important because a low concentration of gelling agent will produce a simple solution or lotion with a very low consistency, whereas a high concentration of gelling agent may form high-viscosity gels that have a low extract distribution uniformity and are difficult to handle.

Fig. 5:- Visual appearance of licorice gel.

5.5 DETERMINATION OF PH

The pH of the formulas (CAR-3 and H-4) was determined for assuring that the formula can be used with no risk of skin irritancy. The pH was determined to be 5.5 ± 0.5 which was close to the required pH for topical preparation, thus the formulas can be used with no risk of skin irritancy. This also indicated that the selected ingredients of the formula did not change the pH of the formula.

5.6 SKIN IRRITANCY TEST:-

After doing this test and observations for any undesirable effects at constant intervals for about 24 h, results represented that there were no redness, no edema and no irritation or other unwanted effects after application.

5.7 SPREADABILITY TEST:-

It was found that the formulations' spreadability decreased as the gelling ingredient's concentration rose. The best gel in this study can be spread with little to no shear force, as indicated by measurements, which place its spreadability value between 0.45 and 0.89 g.cm/sec. The results demonstrated that the formulas (H-4 and CAR-3) can be utilized indefinitely and without experiencing any problems. This ensures that the formulations maintain an appropriate wet contact time when applied to the skin's application site.

5.8 VISCOSITY DETERMINATION:-

Table.5:- Viscosity determination

Formula CAR-3		Formula H-4	
Share force (rpm)	viscosity(mPa.s)	Share force (rpm)	viscosity(mPa.s)
8	16606	8	74289
16	10007.4	16	65989

Regarding viscosity determinations of the two formulas (CAR-3 and H-4), two types of spindle were used. For CAR-3, spindle 3 was used, while spindle 4 was used for H-4. Results showed that Formula H-4 exhibited higher viscosity than formula CAR-3 as demonstrated in table 5.

5.9 STABILITY STUDY FOR THE SELECTED:-

Table.6:- Susceptibility test for 5% licorice gel against different types of microorganisms.

Microorganisms	5 % Licorice gel concentration						Control	
	5	10	15	20	25	30	*Chloram.	**Vorico.
	Zone of inhibition (mm)							
S. aureus	0	14	17	22	27	257	20	--
P. aeruginosa	0	0	0	0	0	0	27	--
C . albicans	10	12	12	22	24	28	-	23

*chloram. = chloramphenicol, **Vorico. = Voriconazoble

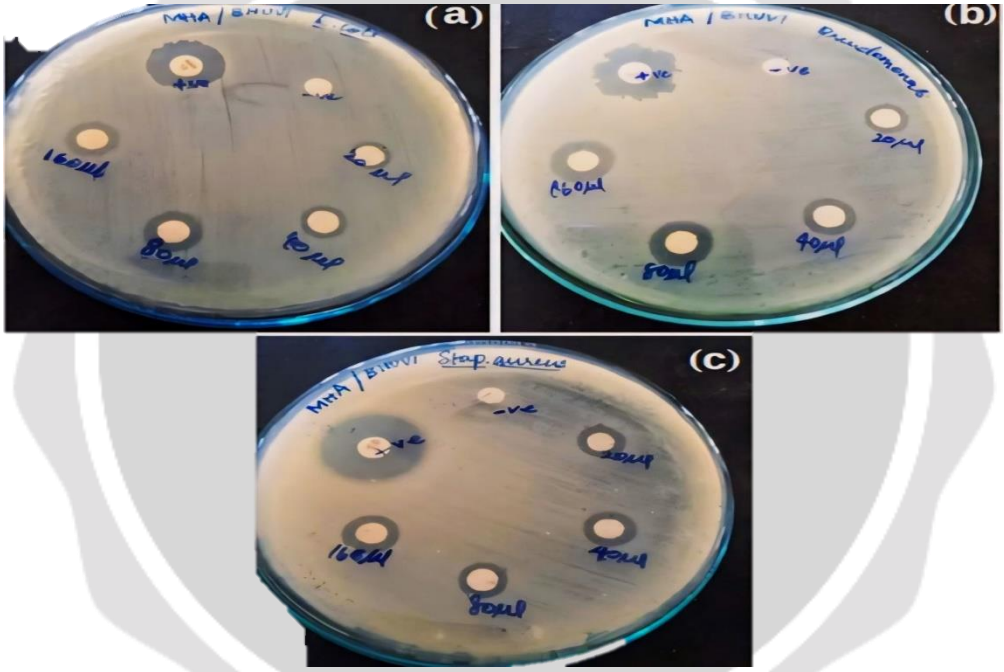
Formulas for Licorice Gel The chosen formulations (CAR-3 and H-4) did not significantly differ ($p \leq 0.05$) in terms of appearance, color, homogeneity, consistency, spreadability, or pH after being tightly sealed and kept at room temperature for four months. This suggested that these mixtures are stable under room temperature storage.

5.10ANTI-MICROBIAL ACTIVITY:-

To examine the antibacterial efficiency against various microorganisms, formula CAR-3 was chosen. Table 6 and present the findings of the anti-microbial activity of 5% licorice gel against S. aureus, P. aureus, and Candida albicans. The anti-microbial activity of 5% licorice gel was shown to be preferable to that of control when it came to S. aureus and C. albicansin. However, no anti-microbial activity was found when it came to P. aeruginosa, a gram negative bacteria which showed increased resistance to the produced gel compared to the yeast and Grampositive bacteria tested (Table 6).

Fig. 6: Susceptability test showed zone of inhibition of 5% licorice gel against S.aureus. S.aeruginosa, and C.albicans.

This could be clarified by the variations in the cell membrane structure.28 According to our findings, The licorice gel that was made



had more antimicrobial efficaciousness against Candida species as opposed to the tested species of bacteria with a zone of inhibition in S. Aureus varied from 0–25 mm, except that for C. albicans, the zone of inhibition was between 12 and30 mm. Positive outcomes were gathered from the work done by Karahana and associates (2016) 29 shown how to use disc agar diffusion Licorice contains antibacterial properties.

activity as well as the inhibitory zone for S. aureus had a mm range of 12–19. P. aerogenosa, however, had no antimicrobial properties. However, there was no antibacterial action for P. aerogenosa. In 2013, Poonam and me demonstrated that the The zone of inhibition for Candida albicans had a diameter ranging from 9 to 20 mm upon treatment with licorice.

5.11ANTI-ULCER ACTIVITY OF LICORICE ROOT:-

The anti-ulcer activity of licorice root was found to be similar to famotidine in the process of indomethacin-induced gastric ulcer in rats. Combination therapy with famotidine and licorice shows higher anti-ulcer activity than either of them alone.

6. CONCLUSION:-

Apparently, licorice gel is easily prepared from ethanol/water licorice extract. Among many formulas, one is selected for further study. Selected gel formulations demonstrate physical and chemical stability including appearance, consistency, pH and skin testing. Additionally, it showed good stability at room temperature without significant changes after four months of storage. The selected formula shows good antibacterial properties against two of the most common causes of skin infections: Gram-positive bacteria (Staphylococcus aureus) and yeast (Candida albicans) and also shows good anti-ulcer action against mouth ulcers. In summary, licorice topical gel can be easily prepared and stored at room temperature. It may be considered a suitable candidate for new herbal antimicrobial and antiulcer pharmaceutical preparation and could be used as an efficient alternative to the conventional topical anti-microbial and oral antiulcer gel preparations to come onto the market.

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