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# Formulation and Evaluation of Herbal Emulgel of hydroalcoholic extract of *Agave tequilana* Trel leaves for Anti-fungal activity.

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**Keywords**

*Agave Tiquilana* Trel leaves,
Herbal emulgel,
hydroalcoholic extract,
Antifungal activity.

## Abstract

Emulgel have emerged as a promising drug delivery system for the delivery of hydrophobic drugs. The objective of the study was to prepare emulgel of Hydroalcohlic extract of *Agave Tiquilana* Trel leave's, using Carbapol 940 as a gelling agent. The emulsion was prepared and it was incorporated in gel base. The formulation was evaluated for antifungal activities along with its rheological studies, spreading coefficient studies, bioadhesion strength, skin irritation, anti-in fungal activity and analgesic activity. Formulation F2 and F4 showed comparable anti-infungal activity. compared with marketed standard formulation. So, it can be concluded that topical emulgel of hydroalcohlic extract of *Agave Tiquilana* Trel Leave's posses an effective anti-in fungal activity.

## 1. Introduction

Emulgel is a combination of emulsion and gel, which is a new approach for topical delivery of drugs. It has a double control release like emulsion and gel. Gel is new class of formulation; it releases the drug faster in comparison ointment, cream and lotion.

Drugs derived from natural sources play a significant role in prevention and treatment of Human diseases In many developing countries, traditional medicine is one of the primary health care systems.[1,2] Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.[3]

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.[4] About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer.[4] recent trends, however, show that the discovery rate of active novel chemical entities is declining.[5] Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc. which have been found in antifungal properties.[6,7] In the current investigation carried out, a screening of emulgel of *Agave tequilana* trel leaves against fungi is done in order to detect new source of antifungal agents.

Blue agaves sprout a tail (quiote) when around five years of age that can grow an extra 5 meters (16 ft); they are finished off with yellow flowers.[8][9] [10]

It is seldom kept as a house plant, however a 50-year-old blue agave in Boston grew a 9 m (30 ft)

Ayurveda is a potential source of indigenous drugs. It is the ancient Indian system of medicine strongly believes in polyherbal formulations and scientists of modern era often ask for scientific validation of herbal remedies, therefore there is a ne d for exhaustive study on the various herbal medicinal plants from Phyto-chemical and pharmacological point of view.

The medicinal herb is a biosynthetic laboratory as it contains number of chemical compounds like glycosides, alkaloids, resins etc. These compounds exert therapeutic effect and account for medicinal property of the medicinal herb.

Medicinal plants are also important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agent, but also when they are used as basic materials for synthesis of drugs or as models for pharmacologically active compounds.

The present work has been performed on leaves of *Agave tequilana* Trel. Different parts of *Agave tequilana* Trel are reported in ayurvedic medicines for valuable treatment of variety of conditions like counter-irritant in rheumatism, neuralgia, headache otalgia, earache and anthelmintic and fungal treatments. Efforts have been made to find out the antifungal activity of the hydroalcholic extract along with screening of emulgel formulation an its evaluation for antifungal activity.

## 2. Plant Profile

**Fig 1 *Agave tequilana* Trel**

### 2.1 Scientific Classification

- Kingdom: Plantae
- *Class* : Liliopsida
- Order : Asparagales
- Family: Asparagaceae
- Genus: *Agave*
- Species: *A.tequilana*

### 2.2 Botanical Name.

*Agave tequilana* Trel

### 2.3 Synonyms

Blue Agave

### 2.4 Description

Agave rosettes are generally monocarpic, however a few animal categories are polycarpic.[7] During blooming, a tall stem or "pole" ("quiote" in Mexico), which can develop to be 12 meters (40 feet) high,[10] develops apically from the focal point of the rosette and bears an enormous number of short, rounded blossoms and in some cases vegetatively delivered bulbils (a type of agamic propagation)..[7,10]

### 2.5 Habitat

The tequila agave is native to the Different states of India. The plant favors altitudes of more than 1,500 metres (5,000 ft) and grows in rich and sandy soils. Blue agave plants grow into large succulents, with spiky fleshy leaves, that can reach over 2 metres (7 ft) in height. Blue agaves sprout a stalk (quiote) when about five years old that can grow an additional 5 metres (16 ft); they are topped with yellow flowers. The stalk is cut off from commercial plants so the plant will put more energy into the heart.

### 2.6 Part Used

Aerial parts of the plant (Leaves)

### 2.7 Traditional use

Aguamiel or agave juice is the yellow sap with herbaceous scent that is obtained from the ripe maguey that contains various sugars principally sucrose and fructose, and other macronutrients such as protein, micronutrients such as amino acids, minerals and vitamins, and also has composites with a potential functional such as the phenolic compounds and saponins.[12]

Agave syrup (commonly called agave nectar), a sweetener derived from the sap, is used as an alternative to sugar in cooking, and can be added to breakfast cereals as a binding agent. The agave sweetener is marketed as natural and diabetic-friendly, without spiking blood sugar levels.

## 3. Materials and Methods

### 3.1 Collection and Drying- [19]

Mature leaves of Agave *tequilana* Trel were collected,From the Hadapsar Industrial estate and cleaned and dried at room temperature in shade and away from direct sunlight. The dried leaves were coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fines and larger particles and the powder was subjected for further study.

**Fig.2 drying of leave**

## 4. Pharmacognostic Study

### 4.1 Macroscopic Study: - [20]

Leaves are grey-green with a size of 0.9-1.5 m (3-5 ft) long that may reach a total height up to 8-9 m (25-30 ft).

**Color**: Dark- Green

**Odor**: Characteristic

**Taste**: Bitter

### 4.2 Microscopic Study:-[20]

Simple and compound starch grains and occasionally few lignified fires are present in the pericyclic region. Few layers of collenchyma were laid under both the epidermis the upper layer located adjacent at the opening of U-shaped xylem. The lower epidermis of midrib bears large number of multiseriate papillose projections. It bears simple covering unicellular trichomes of various sizes containing cystolith at the base. Glandular trichomes with unicellular stalk and bicellular head filed with dark brown content were also present Thin transverse section.

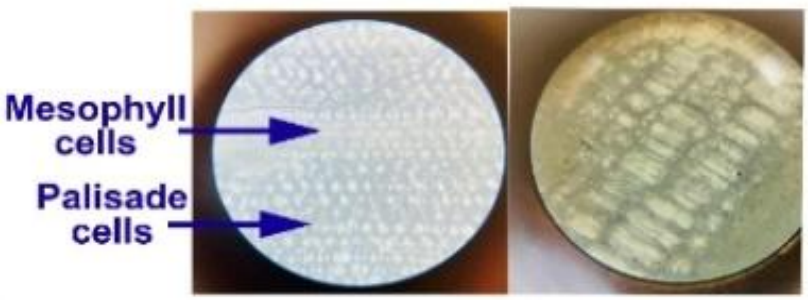



**Fig.3 T.S. of Leaf**

Longitudinal section of middle part of fresh leaf was taken, stained with Phloroglucinol+ HCI concentrated (1:1),observed under microscope in 10X, 45X lenses. The longitudinal section was studied.

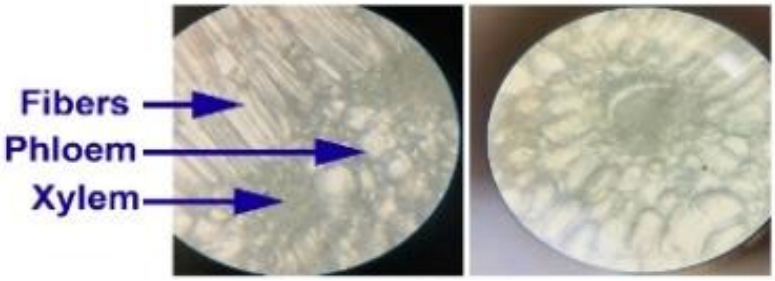



**Fig.4 L.S. of Leaf**

### 4.3 Powder Characteristics [20]

The microscopic examination of powdered leaf material was performed to detect and to establish various peculiar microscopic characters in order to differentiate between the adulterated and the substituted powdered or intact leaves supply.

Slides of powdered leaf material was prepared using formalin, glycerine and water (8:1:1 v/v/V) and were thus embedded and seen under microscope on different magnifications at 10x, 40x, 100x after staining with phloroglucinol and HC1.

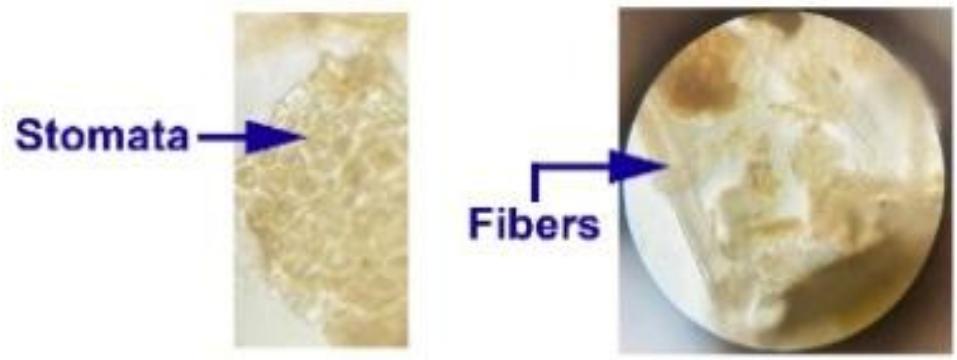



**Fig.5 Powder characteristics**

### 4.4 Determination of loss on drying:-[20]

The powdered drug sample (5 gm) was taken without preliminary drying and was placed on a tarred evaporating dish and dried at 105°C for six hours and weighed.

The drying was continued until two successive reading matches each other or the difference between two successive weighing after drying for 30 minutes in a desicator, showed not more than 0.01 g difference(25).

Accurately weighed a glass-stoppered, shallow weighing bottle was dried. 2g of sample was transferred to the bottle and covered, the weight was taken and sample was distributed evenly to a depth not exceeding 10mm.

Then loaded bottle was kept in the oven and stopper was removed. The sample was dried to Constant weight.

After drying it was cooled to room temperature in a desiccator. Weighed and calculated the loss on drying in terms of percent w/w.

#### Observation

Weights of powdered sample taken = 5 gm
Weights of powdered sample after heating = 1.08 gm

**Result: -** The percentage of loss on drying = 21.6 % w/w

### 4.5 Determination of ash value:[20]

The total ash values, acid insoluble ash value and water soluble ash value were found ot be 14.3% w/w, 4.7% w/w, 10.5% ww/ respectively. Ash value Si useful Ni determining authenticity and purity of drug and also these values are important quantitative standards.

Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes, and inorganic variables like calcium oxalate, silica, and carbonate content of the crude drug affects.

Before ignition of powder:-

•Weight of empty silica crucible = 15.78 gm
•Weight of powder = 2gm
•Weight of silica crucible + powder = 17.78gm

After ignition of powder

• Weight of silica crucible + ash = 16.24 gm
•Total ash. = 0.46 gm

**Results**: - The percentage of total ash obtained = 23%w /w

### 4.6 Extraction Methodology [21, 22]

The solid extraction of drug represents a solid from solid separation. The iquid- liquid extraction is one, in which any of the two immiscible liquids are used for the extraction (Solvent extraction).

Extraction process comes to a halt when the distribution of the extractive substance between miscella and drug residue reaches the value 'K', ie. when the concentration gradient between miscella and reduces has become zero.

Concentration of extracted substances in the miscella

K=Concentration of extractive substance in the drug residue.

### 4.7 Percentage yield of Extract:-[20]

It indicates the approximate measures of the chemical constituent plant. All the values were taken in triplicate and the mean average was taken**.**

50 gms of the powdered drug was taken in a weighing bottle and transfer it in 500 ml graduated flask was filled with the solvent (90 % alcohol).

Flask was closed and set aside for 24 hours, shaking frequently. (Maceration) extract was filtered into a 50 ml cylinder.

When sufficient filtrate has collected, 100 ml. of the filtrate was and Evaporated to dryness on a water-bath and complete the drying in an oven at 100° C.it was Cool in a desiccator and weigh.

Percentage w/w of extractive was calculated with reference to the air-dried drug.

Weight of powdered leaves taken. = 50 gm
Weight of Extract obtained = 2.5 gm

**Result:** Percentage yield of Hydro-alcoholic Extract was obtained = 5.0 % w/w

### 4.8 Preliminary Phytochemical Screening

#### 1.Test for Steroids [23]

#### a) Salkowski test

One mI of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown colour exhibited by chloroform laver and green fluorescence by the acid layer suggests the presence of steroids.

#### b) Liebermann test

2 mg of the residue few ml of acetic anhydride was added and gently heated. The contents of the test tube were cooled and 2 ml of concentrated sulphuric acid was added from the side of the test

tube. Development of blue colour gave the evidence for presence of sterols.

**c) Liebermann-Burchard test**

10 mg extract was dissolved in 1ml of chloroform and 1ml ofacetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the sides of the test tube. Formation ofreddish violet colour at thejunction indicates the presence of steroids.

**2.Test for Saponins.[24,25]**

**a) Foam formation test**

One mi solution of the extract was diluted with distilled water to 20 mlAlid shaken in a graduated evlinder for 15 minutes. The development of stable foam indicates the presence of saponins.

**3. Test for Alkaloids**[24,25]

**a) Dragendorff's test**

01 ml dilute hydrochloric acid and 0.1 ml Dragendorff's reagent wasadded in 2 ml of extracts in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

**b) Mayer's test**

02 ml of extract was taken in test tube. 0.2 ml of dilute hydrochloric acidand 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

**c) Wagner's test**

02 ml of extract was treated with 0.2 ml dilute hydrochloric acid and 0.1 mlof Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

**4. Test for Glycoside.[26]**

**A) Modified Borntrager's test**

The extract was treated with ferric chloride solution and heated on a Dolling water bath for 5 min. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. The formation of rose pink or cherry red colour in ammoniacal layer indicates the presence of anthraquinones.

**b) Millon's test**

The extract was treated with 2 ml of Millor's reagent. The formation of wile precipitate, which turns to red upon heating indicates the presence of proteins and amino acids.

**5. Test for Flavonoids**[25,27]

Few drops of 10 per cent lead acetate are added to the extract development of yellow ppt confirms the presence of flavonoids.

**6. Test for Tannins [20]**

**a) Ferric Chloride test**

Five ml of extract solution was allowed to react with 1 ml of 5 per cent ferric chloride solution. Greenish black colouration indicates the presence of tannins.

**b) Lead Acetate test**

Five ml of extract was treated with I ml of 10 per cent aqueous lead acetate solution. Development of yellow coloured precipitate indicates the presence of tannins.

**c) Potassium Dichromate test**

Five ml of extract was treated with 1 ml of 10 per cent of aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggests the presence of tannins.

**7. Test for Proteins [25]**

**a) Buret test**

The extract was treated with 1 ml of 10 per cent sodium hydroxide solution and heated. A drop of

0.7 per cent copper sulphate solution was added to the above mixture. The formation of purplish violet colour indicates the presence of proteins.

### b) Xanthoproteic test

A little test residue was taken in 2 ml of water and to it 5 ml of concentrated nitric acid was added. Formation of yellow colour indicates tine presence of proteins.

### c) Million's test

The extract was treated with 2 ml of million's reagent. The formation of site precipitate, which tuns to red upon heating, indicates the presence of proteins and amino acids.

## 8. Test for Amino Acids.[25]

### a) Ninhydrin test

The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Development of purple colour indicates the presence of aminoacids.

## 9. Test for Carbohydrates.[28]

### a) Molish test

Two ml of extract solution was treated with few drops of 15 per cent ethanolic alpha-naphthol solution in a test tube and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. The formation of a reddish violet ring at the junction of two layers indicates the presence of carbohydrates

### b) Barfoeds test

The test residue was dissolved in water and heated with a little quantity of Barfoed's reagent. Development of brick red precipitates within two minutes shows the presence of monosaccharide.

## 10. Test for reducing sugars [28, 29]

### A) Felhing’s test

05 ml of extract solution was mixed with 5 ml of Fehling's solution equal mixture of Fehling's solution A&B) & boiled. Formation of brick red Precipitate indicates the presence of reducing sugars.

### b) Benedict's test

Equal volumes of Benedict's reagent and extract ni test tube and boiled for S min solution appeared green, yellow or red depending upon the amount of reducing sugar present.

**Table1. Preliminary Phytochemical Screening**

| Chemical tests | Hexane Extract | Hydroalcoholic Extract |
|---|---|---|
| Test for Steroids | - | + |
| Test for Saponins | + | + |
| Test for Alkaloid | - | + |
| Test for Glycosides | - | - |
| Test for Reducing sugars | - | - |
| Test for Tannins | - | + |
| Test for Flavonoids | + | + |
| Test for Amino acids | + | - |
| Testfor Carbohydrates | + | + |

## 5. Screening of extract for antifungal activity

**Antifungal Assay**

Three fungal strains (*Aspergillus niger* , Rhizopus, Mucor) These strains have been selected for the basis of its application purpose of further formulation study. Anti fungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antifungal activities are presented in Tables.

**Table: 2 Antifungal activities of standard drugs against fungal test organism.**
**(Zone** of inhibition in mm)

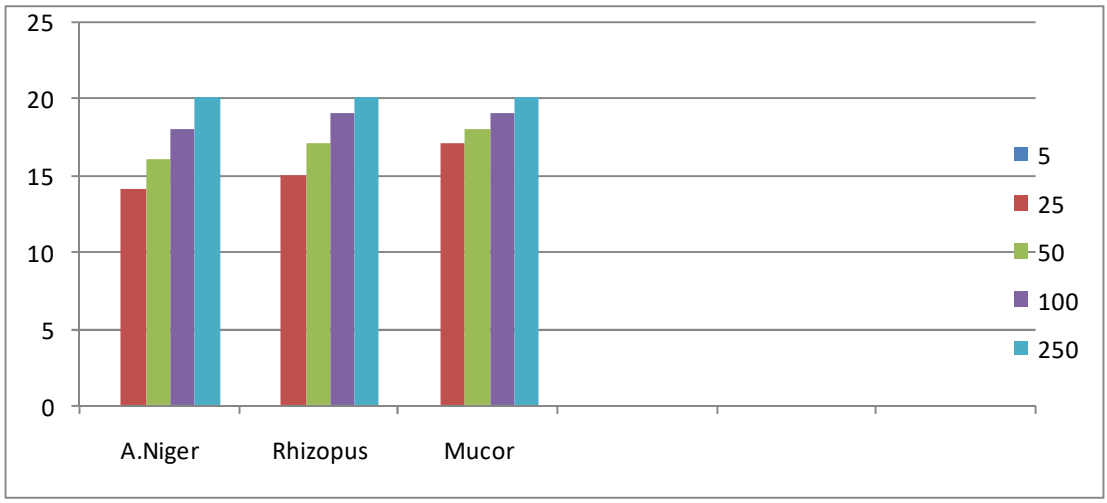
| Drug | concentration( µg/ml) | *A.niger* | *Rhizopus* | *Mucor* |
|---|---|---|---|---|
| **Grisofulvin** | 25 | 23 | 21 | 21 |
| | 50 | 25 | 22 | 22 |
| | 100 | 25 | 23 | 24 |
| **Nystatin** | 25 | 18 | 21 | 21 |
| | 50 | 24 | 24 | 24 |
| | 100 | 29 | 26 | 25 |

### Antifungal activities of Hydro- Alcoholic Extract.

Antifungal activities of the extracts increased linearly with increase in concentration of extracting (µg/ml). As compared with standard drugs, the results revealed that in the extracts for fungal activity, Rhizopus shows good result as compare with *Aspergillus niger* and Mucor The growth inhibition zone measured ranged from 11 to 20mm for all the sensitive bacteria, and ranged from 14 to 20 mm for fungal strain.

**Table: 3 Antifungal activity of Hydro-Alcoholic Extract**
**(Zone of inhibition in mm)**

| concentration in ( µg/ml) | Aspergillus niger | Rhizopus | Mucor |
|---|---|---|---|
| **5(µg/ml)** | 0 | 0 | 0 |
| **25(µg/ml)** | 14 | 15 | 17 |
| **50(µg/ml) (µg/ml)** | 16 | 17 | 18 |
| **100(µg/ml)** | 18 | 19 | 19 |
| **250(µg/ml)** | 20 | 20 | 20 |




**Different concentration of hydroalcoholic extract.**
**Fig.8 Zone of Inhibition**

## 6. Formulations of Emulgel

The gel portion of the emulgel was made by dissolving carbopol-934 in cold water with constant stirring at a moderate speed until uniform mixture was made. The pH was then adjusted to 6-6.5 using triethanolamine (TEA. Tween 80 was dissolved in distilled water to prepare the aqueous phase of the emulsion while for the preparation of the oil phase of the emulsion, span 80 was dissolved in liquid paraffin. To preserve the emulsion, methyl parabene was dissolved in propylene glycol and the extract was dissolved in ethanol then both solutions were mixed with the aqueous phase. Both the aqueous and the oil phase were heated in a water bath at 70 °C separately. Then the oil phase was added drop wise to the aqueous phase with continuous stirring using homogenizerat speed of 3000 pm for 10 min then cold to room temperature.[30]

At the end the gel and emulsion portions were mixed in 1:1 ratio with moderately stirring to prepare emulgel.

**Table 4. Formulations of Emulgel**

| Sr.no | Ingradient | Quantity Taken |
|---|---|---|
| 1 | Hydoalcoholic extract | 5gm |
| 2 | carbopol 934 | 0.75 gm |
| 3 | Span 60 | 0.45 ml |
| 4 | Tween 80 | 0.50 ml |
| 5 | Liquid paraffin | 2.5 ml |
| 6 | Propylene glycol | 3.5 ml |
| 7 | Methyl paraben | 0.01 gm |
| 8 | Water | q.s. |

## 7. Evauation of emulgel formulation.

### A. Physical Evaluation:

Physical parameters such as color and appearance were checked.

### B. Measurement of pH:

pH of the gel was measured by using pH meter.

### C. Viscosity;

Viscosity of gel was measured by using Brookfield viscometer with spindle.

### D. Spreadibility :

Spreadibility was determined by the apparatus which consists of a wooden black,which was provided by a pulley at an end. By this method spreadibility was nearest on the basis of slip and drag characteristics of Emulgel. An excess of Emulgel (about 2g) under study was placed on the ground slide. The Emulgel was then sandwiched between this slide and another glass side having the dimension of fixed ground slide and provided with the hook.

A one kg weighted was placed on the top of the two sides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5cm be noted. A shorter interval Indicate better spreadibilty. Spreadability was calculated using the following formula:

**S=M=L/T**

Where,
S=Spreadability,
M-Weight in the pan (tied in the upper slide)
1-Length moved by the glass side
T=Time (in sec.) takes to separate the să de completely each other.

### E. Stability Study :

The stabilty study was performed as per ICH guidelines & The Formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, vix 250 C/20C/ 60% 5% RH 300 C 20C/65% /5% RH 400 C20C/ 75% 15% RH for a period of three months and studied for appearance, pH, and spreadability.

### F. Greasiness

Here the Emulgel was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like.

According to the results, we can say that all three formulations were non-greasy.

### G. Irritancy

Mark the area (1 cm?) on left hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and edema if any for an interval up to 24 h and reported. According to the results all the three formulations that is F1H, F2H and F3H showed no sign of irritancy, erythema and edema.

## 8. Results and Discussion

*Agave tequilana* Trel leaves under microscope T.S shows mesophyll cells, palisade cell. L.S. shows Xylem Phloem and Fibres, poweder shows Fibres and Stomata.

*Agave tequilana* Trel leaves after drying calculation of loss of drying was to be obtained 21.6% w/w and powder under muffle furnance gives ash Value of 23% w/w.

*Agave tequilana* Trel leaves after maceration extraction and constant stirring in water bath gives the product, that product was calculated 5% w/w to be obtained.

*Agave tequilana* Trel showing Preliminary Phytochemical screening Hydro- Alcoholic Extract shows presence of Steroids, Saponins, Tannins, Flavonoids. Benzene Extract shows presence of Steroids, Saponins, are present.

## 9. Conclusion

Hydro-alcoholic Extract of *Agave tequilana* Trel leaves showed prominent anti-fungal activity against human pathogenic, thus *Agave tequilana* Trel leaves can be used as a potential antifungal drug against human pathogenic fungal infection.

By using *Agave tequilana* Trel leaves extract Emulgel showed a Antifungal effect. Based on results and discussion, the formulations F1H, F2H and F3H were stable at room temperature and can be safely used on the skin.

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