# PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF *TRIGONELLA FOENUM-GRAECUM* LEAVES EXTRACTS FOR ITS WOUND HEALING ACTIVITY

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***Abstract*: The present study reports physicochemical characterization, antioxidant, antimicrobial and Wound Healing activity of extracts from *Trigonella Foenum-graecum* leaves collected from local region of Nanded, Maharashtra, India. The extracts were obtained from Soxhlet method by using ethyl acetate and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed.**

**The presence of primary and secondary metabolites such as carbohydrate, amino acids, tannins, alkaloids, phenolic compounds, saponins were confirmed through preliminary phyto-chemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of Ethyl acetate and methanol leaves extracts. Maximum percentage inhibition i.e. 80.97% was shown by Ethyl acetate extract at concentration of 150 µg/ml and was compared with Ascorbic acid as reference standard.**

**The *in vitro* Antibacterial property of *Trigonella Foenum-graecum* leaves was carried out by using agar cup and plate method. In this method, increase in zone of inhibition was Ethyl acetate extract having better antibacterial activity on *Baccilus substillus* than *Staphylococcus aureus* & *E.coli.* Methanol extract having better antibacterial activity against *Baccilus substillus* & *Staphylococcus aureus* than *E.coli***

**Antifungal property of *Trigonella Foenum-graecum* leaves was carried out by using poison plate method. In this method, reducing growth of fungi (moderate antifungal activity) and no growth of fungi of test sample was calculated and compared with standard i.e. (*Griseofulvin*). Both extract showed the reduced growth (more than 50% and less than 90% reduction in growth) at 100 mg/ml.**

**The *In-Vivo* Wound Healing activity of *Trigonella Foenum-graecum* leaves was evaluated by excision wound model in rats using Soframycin as standard. Both the extracts at 5 % conc$^n$ showed significant reduction in wound size.**

**The result suggest that *Trigonella Foenum-graecum* leaves extracts possess wound healing activity and this might be due to flavonoids, Phenolic compound, coumarin, tannin and saponins present in extract.**

***Keywords*: *Trigonella Foenum-graecum*, Ethyl acetate and Methanolic extract, Phytochemical screening, Antioxidant effect, Anti-microbial activity, Wound healing activity.**

## Introduction

Wound may be defined as a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may be produced due to physical, chemical, thermal, microbial or immunological exploitation to the tissues. Wound healing is a process of restoring normal structure and functions of damaged tissue. Healing is a natural phenomenon by which body itself overcome the damage to the tissue but the rate of healing is very slow and chance of microbial infection is high. This creates demand of a substance that speeds up the rate of healing.

Wound healers are one of the most critical requirement in the essential medicaments for soldier and may help in putting injured soldier back on the war field as quickly as possible. A wound healer also minimizes demand of other drugs like antibiotics and also their probable side effects by their use. India has a rich tradition of plant-based knowledge on healthcare. A large number of plants/plant extracts/decoctions or pastes are equally used by tribal and folklore traditions in India for treatment of cuts, wounds, and burns. Besides this, there is not a single synthetic drug formulation in the market which can claims for its wound healing properties. The drugs available are either bacteriostatic or bactericidal and in these cases healing is by a natural phenomenon only.

**1. The inflammatory phase:** The inflammatory phase starts immediately after the injury that usually last between 24 and 48 hrs may persist for up to two weeks in some cases. The inflammatory phase launches haemostatic mechanisms to immediately stop blood loss from the wound site.

**2. The fibro plastic phase:** The second phase of wound healing is the fibro plastic that last up to two days to three weeks after the inflammatory phase. The phase comprises of three steps viz., granulation, contraction and epithelialization.

**3. Remodeling phase:** This phase last for 3 weeks to 2 years. Tissue tensile strength is increased due to intermolecular cross linking of collagen via vitamin c dependent hydroxylation.

The rapidity of wound healing depends to a considerable extent on the contraction that begins a few days after injury and continuous for several weeks.

Fenugreek, *Trigonella Foenum-Graecum Linn*, is an annual herb of bean family, reaching 30-60 cm and largely cultivated in India, Egypt and Morocco. The name fenugreek comes from ***foenum-graecum***, meaning 'Greek hay', as the plant was traditionally used to scent inferior hay and the name of the ***Trigonella*** is derived from the old Greek name, denoting 'three angled', probably referring to the triangular shape of flowers.

Fenugreek has strong flavor and aroma. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriented countries as a spice in food preparations and as a ingredient in traditional medicine.

Medicinally it was used for the treatment of wound abscesses, arthritis, bronchitis, ulcer and digestive problems. The plant grows to height of about 3 feet. ***Trigonella Foenum-graecum Linn*** has long stalked leaves up to 5cm long stipules triangular; lanceolate leaflets about 2.5 cm long. The root mass of fingery structure. Flowers are white and pale yellow. The plant radiates spicy odor which persist on the hands after touching. Fenugreek is best grown as a annual crop from seeds by the line sowing method.

The leaves contain 7 saponins, known as *graecunins*. These compounds are glycosides of diosgenin. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1% and carbohydrate 6% & the mineral and vitamins (calcium, iron, phosphorous, carotene, thiamine, riboflavin, niacin, and vitamin c).

## MATERIAL AND METHODS

**1.Collection, Identification and Authentication of Plant Material:**

The fresh Leaves of *Trigonella Foenum-graecum* was collected from local region of Nanded i.e. from local market and authenticated by **Dr. Shrirang S. Bodke,** Head, Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded**.**

**2.Processing of plant material:**

Shade drying of the leaves up to complete removal of moisture was done. (Took around 15 days). Dried leaves were powdered by hand crushing and sieved through sieve number 30.

**3. Prepration of Extract**

Two extracts of *Trigonella Foenum-graecum* leaves powder were prepared.

- **Ethyl Acetate extract**
- **Methanolic extract**

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$(\%)\ \text{yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

**Preparation of Ethyl Acetate extract:**

Ethyl Acetate extract of powdered leaf was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 209 gm of the powdered leaf and1400 ml Ethyl Acetate was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

**Preparation of Methanolic extract:**

Methanolic extract of powdered leaf was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 209 gm of the powdered leaf and 1400 ml Methanol was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

**Phytochemical Evaluation:**

**1. Total Phenolic Content**

Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1ml) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of **Folin-Ciocalteu** phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCO_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

**2. Total Flavonoids Content**

Total Flavonoid Content was measured by the aluminium trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

**3. *In-vitro* Antioxidant activity:**

**DPPH radical scavenging assay:**

The measurement of radical scavenging activity of any antioxidant is commonly associated with the using of DPPH method because it is quick, reliable and reproducible method.

The free radical scavenging activity of the compound was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as per standard reference.

**Principle:**

The assay is based upon the theory that $H^+$ is antioxidant. The antioxidant effect is proportional to the disappearance of DPPH in test sample due to which purple solution changes to yellow.

The degree of color change from purple to yellow at different concentrations was spectrophotometrically measured at 516 nm. The degree of discoloration indicated the scavenging potential of the antioxidant compounds in the term of hydrogen donating ability.

**Procedure:**

10µg/ml to 50µg/ml of both test i.e. rutin as well as standard i.e. ascorbic acid were prepared in methanol along with that 100µg/ml of DPPH. 2ml of various concentrations (10µg/ml - 50µg/ml) of rutin as well as ascorbic acid were added to 2 ml solution of 100 µg/ml DPPH. An equal amount of methanol and DPPH served as control. After 30 min of incubation at room temperature in the dark, the absorbance was recorded at 516 nm.

$$\% \text{ DPPH radical scavenging activity} = 1 - [A_{sample}/A_{control}] \times 100$$

Where,

$A_{sample}$ and $A_{control}$ are absorbance of sample and control

**4. *In vivo* Wound Healing activity**

**Principle:**

The rats were inflicted with excision wounds as described by Morton and Malone (1972) using Ketamine.

**Animal used:**

For the study, *Wistar rats* of either sex, of weight 150-200 gm were selected.

**Test group:**

For the study, seven groups of animals were made; each group having six rats.

**Route of administration:** Topical administration.

**Animal Grouping and drug administration:**

*Wistar rats* of either sex weighing 150-200 gm, obtained from animal house of college. The Animals were randomly divided into seven groups of six animals in each group namely:-

1) **Positive Control**: Treated with plain ointment base
2) **Negative Control**: Non treated
3) **Standard**: Treated with standard drug, i.e., Soframycin
4) **Test Dose-1**: Treated with 2% ethyl acetate extract (TFG EA 2%)
5) **Test Dose-2**: Treated with 5% ethyl acetate extract (TFG EA 5%)
6) **Test Dose-3**: Treated with 2% methanolic extract (TFG M 2%)
7) **Test Dose-4**: Treated with 5% methanolic extract (TFG M 5%)

**Procedure:**

- The animals were divided into Seven groups with six in each were anaesthetized by open mask method with Ketamine before wound creation.
- The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 $mm^2$ full thickness of skin from a predetermined shaved area.
- The wound was left undressed to the open environment. The ointment base, standard drug ointment and extract of plant ointment (2%, w/w) & (5%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound get completely healed.
- In this model, wound contraction was monitored.
- Wound contraction was measured as percent contraction in each 2 days after wound formation.
- From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.

**Evaluation**

An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wound was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 $mm^2$, as 100%, by using the following formula:

$$\% \text{ wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

**RESULTS**

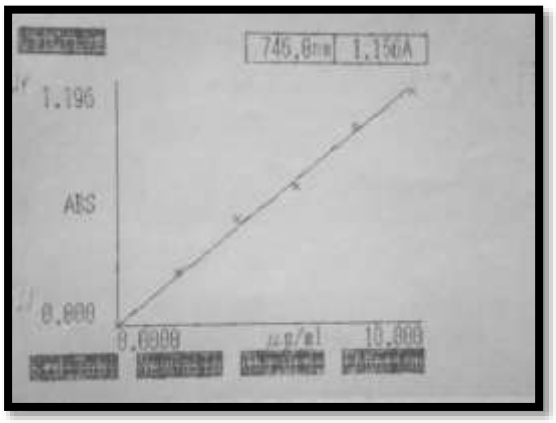
**1. Observations for Phytochemical qualitative analysis**

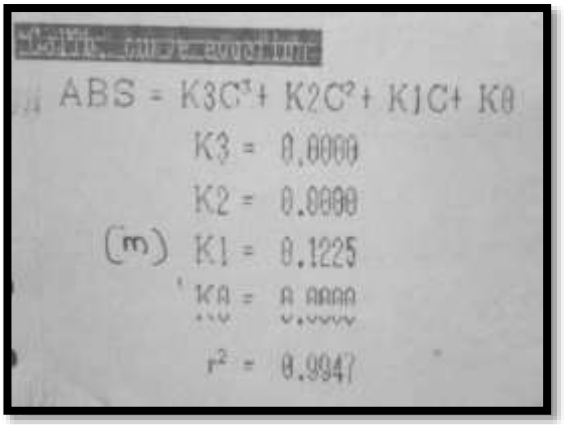
| Sr. No. | Tests | Petroleum ether extract | Ethyl Acetate extract | Methanolic extract |
|---|---|---|---|---|
| 1 | Carbohydrate | – | + | + |
| 2 | Proteins | – | – | – |
| 3 | Amino acids | + | + | + |
| 4 | Glycosides | + | + | + |
| 5 | Alkaloids | – | + | – |
| 6 | Flavonoids | – | + | + |
| 7 | Phenolic compounds | – | + | + |
| 8 | Tannins | + | + | + |
| 9 | Saponins | + | + | + |
| 10 | Fats and oil (Fixed oil) | + | + | – |
| 11 | Volatile oil | – | + | + |
| 12 | mucilage | + | + | + |
| 13 | vitamins | + | + | + |
| 14 | Coumarins | – | + | + |
| 15 | Phytosterols | – | – | + |

**2. Estimation of Total Phenolic Content**

| Sr. No. | Concentration (µg/ml) | Absorbance | | |
|---|---|---|---|---|
| | | GA | Ethyl acetate | Methanol |
| 1 | 2 | 0.266 | 0.231 | 0.201 |
| 2 | 4 | 0.534 | 0.424 | 0.496 |
| 3 | 6 | 0.702 | 0.75 | 0.72 |
| 4 | 8 | 1.013 | 0.929 | 0.896 |
| 5 | 10 | 1.156 | 1.056 | 1.06 |

**2.1 Calibration Curve of Gallic acid**




**2.2 Results of Total Phenolic Content**

| Sr. no | Extract | Concentration (µg/ml) | Absorbance | TPC mg/g of GAE |
|---|---|---|---|---|
| 1 | Eth. A. Extract | 6(µg/ml) | 0.750 | 0.976 |
| 2 | Meth. Extract | 8(µg/ml) | 0.896 | 0.966 |

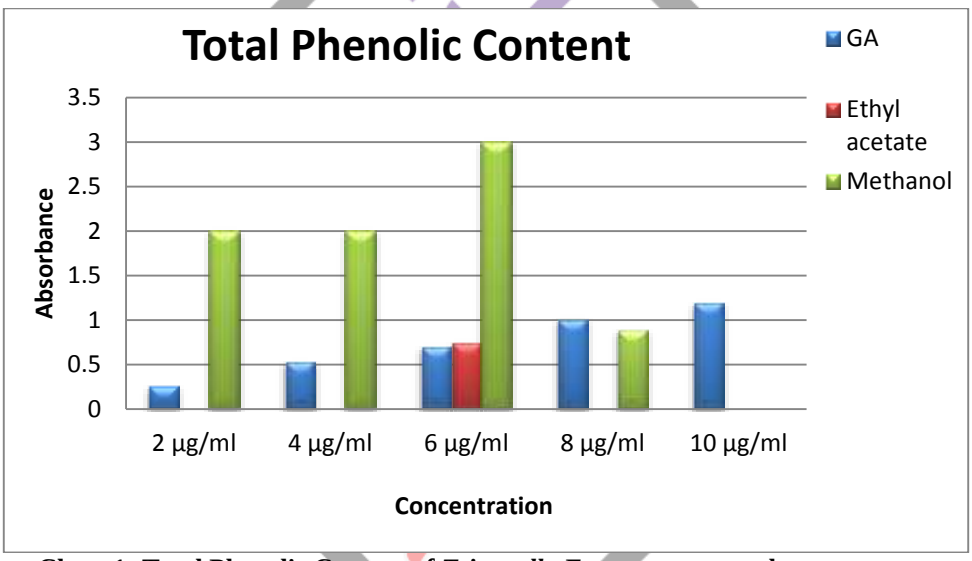
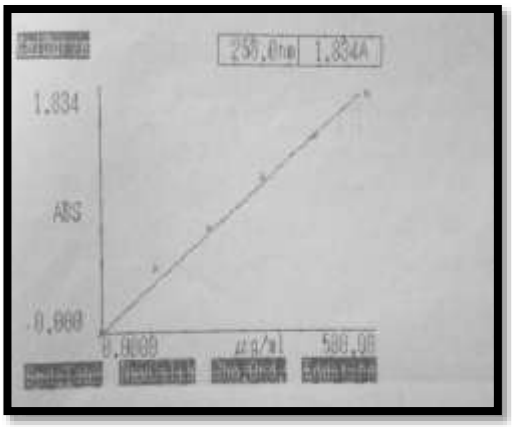


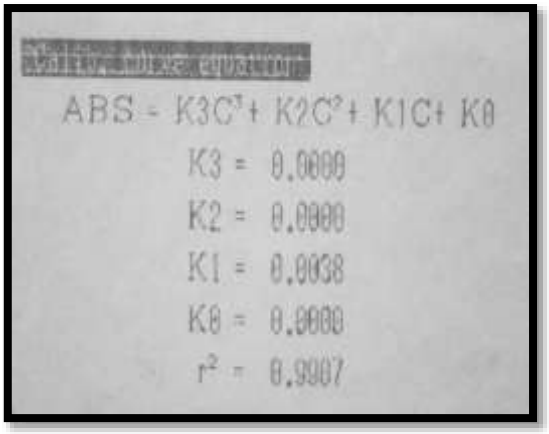

**Chart 1: Total Phenolic Content of *Trigonella Foenum-graecum* leaves extracts**

**3. Estimation of Total Flavonoid Content**

| Sr. No. | Concentration (µg/ml) | Absorbance | | |
|---|---|---|---|---|
| | | Rutin | Ethyl Acetate | Methanol |
| 1 | 50 | 0.49 | 0.35 | 0.399 |
| 2 | 100 | 0.791 | 0.598 | 0.65 |
| 3 | 150 | 1.2 | 1.036 | 1.039 |
| 4 | 200 | 1.505 | 1.323 | 1.37 |
| 5 | 250 | 1.834 | 1.685 | 1.701 |

### 3.1 Calibration Curve of Rutin




### 3.2 Results of Total Flavonoid Content

| Sr. no | Extract | Concentration (µg/ml) | Absorbance | TFC mg/g of Rutin |
|---|---|---|---|---|
| 1 | Eth. A. Extract | 140 (µg/ml) | 1.038 | 9.333 |
| 2 | Meth. Extract | 100 (µg/ml) | 0.650 | 8.666 |

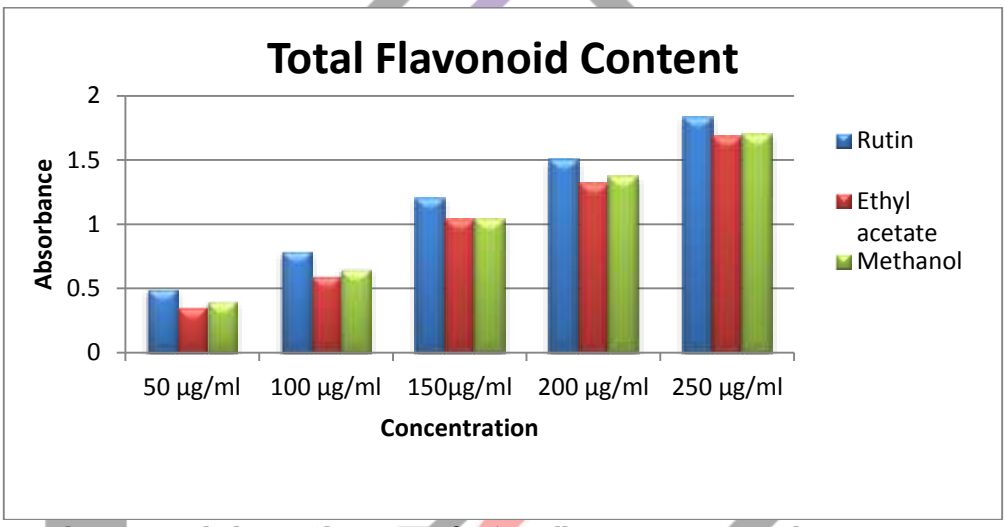


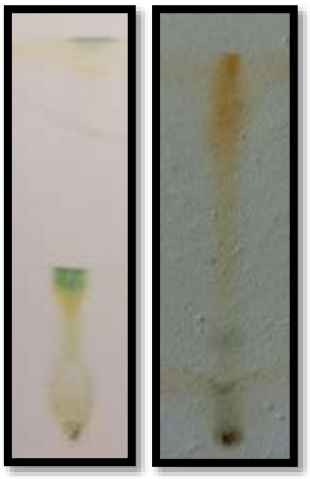

**Chart 2: Total Flavonoid Content of *Trigonella Foenum-graecum* leaves extracts**

### 4. TLC Fingerprinting

| Sr. No. | Extracts | Solvent systems | Proportions | Spraying Reagent | $R_f$ |
|---|---|---|---|---|---|
| 1. | Ethyl Acetate extract | Chloroform: Glacial acetic acid: Ethanol | (3:6:3) | Sulphuric acid | 0.42 |
| 2. | Methanolic extract | Ethyl acetate: Chloroform: Ethanol | (3:3:6) | Sulphuric acid | 0.52<br>0.78 |

**Ethyl Acetate** extract of *Trigonella Foenum-graecum* when subjected to TLC fingerprinting showed $R_f$ value at 0.42, in the solvent system of Chloroform: Glacial acetic acid: Ethanol (3:6:3).

**Methanolic** extract of *Trigonella Foenum-graecum* when subjected to TLC fingerprinting showed $R_f$ value at 0.52, 0.78, in the solvent system of Ethyl acetate: Chloroform : Ethanol (3:3:6).



**Ethyl acetate extract** **Methanolic extract**

**5. Antioxidant activity**

**5.1 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity of Ethyl Acetate extract of *Trigonella Foenum-graecum* leaves**

| Sr. No. | Conc. (µg/ml) | Absorbance of Ethyl acetate extract of leaf | % Inhibition of Ethyl acetate extract of leaf | Absorbance of Standard (A.A.) | % Inhibition of Standard (A.A.) |
|---|---|---|---|---|---|
| 1 | 50 | 0.341±0.002 | 38.04 | 0.312±0.002 | 61.95 |
| 2 | 100 | 0.140±0.003 | 64.87 | 0.040±0.001 | 95.12 |
| 3 | 150 | 0.176±0.001 | 80.97 | 0.031±0.002 | 96.22 |

**5.2 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity of methanolic extract of *Trigonella Foenum-graecum* leaves**

| Sr. No. | Conc. (µg/ml) | Absorbance of Methanolic extract of leaf | % Inhibition of Methanolic extract of leaf | Absorbance of Standard (A.A.) | % Inhibition of Standard (A.A.) |
|---|---|---|---|---|---|
| 1 | 50 | 0.361±0.002 | 22.43 | 0.312±0.002 | 61.95 |
| 2 | 100 | 0.255±0.001 | 68.29 | 0.040±0.001 | 95.12 |
| 3 | 150 | 0.196±0.008 | 74.14 | 0.031±0.002 | 96.22 |

**5.3 Comparative DPPH Scavenging assay method of *Trigonella Foenum-graecum* (Ethyl acetate and Methanolic) leaves extracts**

| Sr. no | Concentration (µg/ml) | As. acid (% inhibition) | Ethyl acetate extract (%inhibition) | Methanolic extract (%inhibition) |
|---|---|---|---|---|
| 1 | 50 | 61.95 % | 38.04 % | 22.43 % |
| 2 | 100 | 95.12 % | 64.87 % | 68.29 % |
| 3 | 150 | 96.22 % | **80.97 %** | **74.14 %** |

**6. Anti- fungal activity**

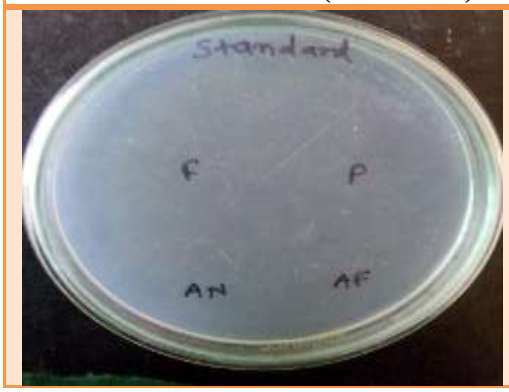
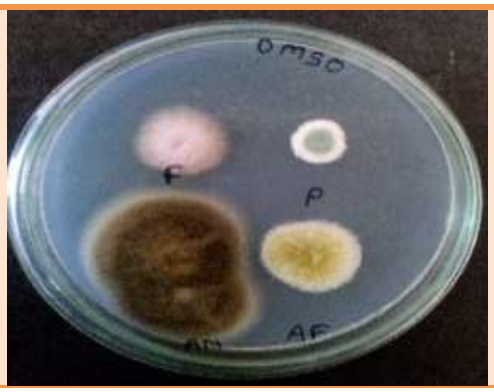
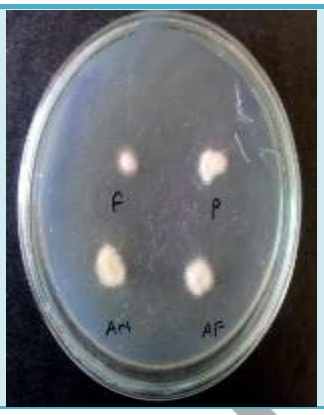
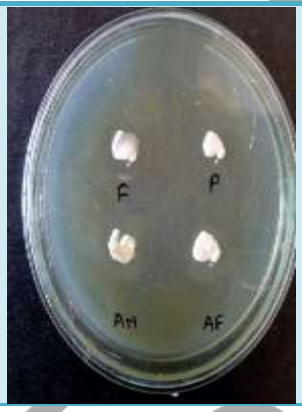
| Effect of reference standard (Griseofulvin) | Effect of DMSO |
|---|---|
|  |  |



**Image : Effect of extracts at different concentration**

| TFG 1 | TFG 2 | TFG 11 | TFG 21 |
|---|---|---|---|
|  |  |  |  |



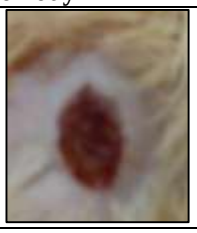
**6.1 Evaluation of Anti-Fungal Activity:**

| Sr. No. | Dose (mg) | Compound | Aspergilus niger | Penicillium chrysogynum | Fusarium moneliforme | Aspargillus Flavus |
|---|---|---|---|---|---|---|
| **1.** | 100 | TFG 1 | RG | RG | RG | + VE |
| **2.** | 100 | TFG 2 | RG | RG | RG | RG |
| **3.** | 50 | TFG 11 | RG | RG | RG | +VE |
| **4.** | 50 | TFG 21 | RG | RG | RG | RG |
| **5.** | 100 | DMSO | + VE | + VE | + VE | +VE |
| **6.** | 100 | Griseofulvin | −VE | −VE | −VE | -VE |

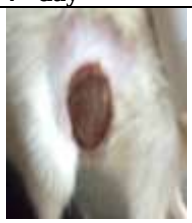
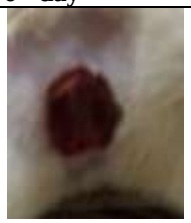
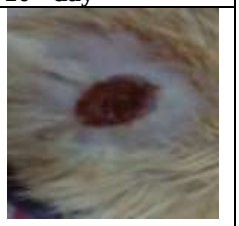
***7. In-Vivo* Wound healing activity**

**Day to day progress of wound healing effect:**

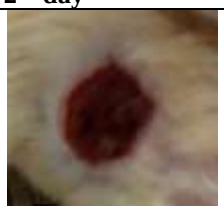
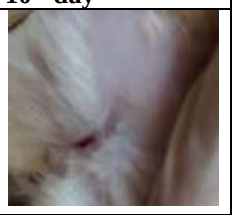
1. **Positive control group:**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

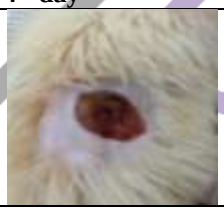
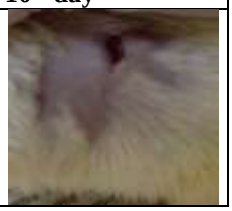
2. **Non-treated group:**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

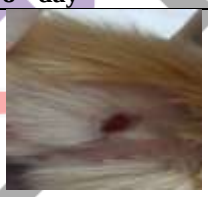
3. **Standard group:**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

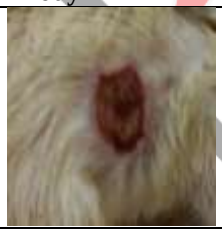
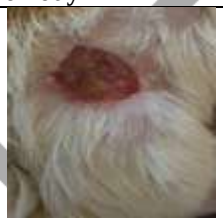
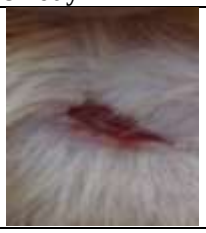
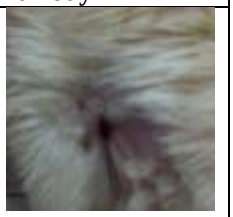
4. **TFG- Ethyl acetate 2% :**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

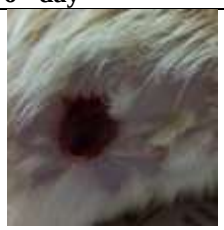
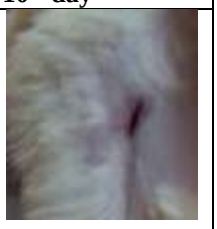
5. **TFG- Ethyl acetate 5% :**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

6. **TFG- Methanol 2% :**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

7. **TFG- Methanol 5% :**

| 0 day | 2nd day | 4th day | 6th day | 8th day | 10th day |
|---|---|---|---|---|---|
|  |  |  |  |  |  |

**7.1 Evaluation of wound healing activity of *Trigonella Foenum-graecum* leaves**

| Groups | Wound area (cm) Post wounding days | | | | | |
|---|---|---|---|---|---|---|
| | 0 Day | 2nd Day | 4th Day | 6th Day | 8th Day | 10th Day |
| Positive control (Group-1) | 2±0.00 | 1.82±0.01 | 1.7±0.02 | 1.5±0.02 | 1.21±0.00 | 1±0.01 |
| Negative control (Group-2) | 2±0.00 | 1.9±0.01 | 1.8±0.01 | 1.5±0.01 | 1.15±0.00 | 1±0.00 |
| Standard (Group-3) | 2±0.00 | 1.7±0.03# | 1.3±0.04* | 1±0.04** | 0.68±0.00** | 0.06±0.02** |
| TFG EA-2% (Group-4) | 2±0.00 | 1.8±0.02#, # | 1.5±0.02#, # | 1.2±0.02**,# | 0.7±0.00**, # | 0.1±0.03**, # |
| TFG EA-5% (Group-5) | 2±0.00 | 1.7±0.03#, # | 1.1±0.10**,#,▫ | 1±0.04**,#,▫ | 0.1±0.08**,**,▫ | 0.05±0.03**, #,▫ |
| TFG M-2% (Group-6) | 2±0.00 | 1.8±0.02#,# | 1.3±0.03*, # | 1.2±0.03**,* | 0.49±0.00**,*,▫ | 0.1±0.00**, # |
| TFG M-5% (Group-7) | 2±0.00 | 1.6±0.04#,#,▫ | 1.2±0.09**,#,▫ | 1.1±0.02**,# | 0.28±0.01**,**,▫ | 0.06±0.00**, #,▫ |

*(*Significant difference when standard and test compared with positive control (P < 0.05);*
*** Highly significant difference when test compared with positive control (p< 0.001);*
*# No significant difference when test compared with standard;*
*▫ Significant difference when test compared with standard. )*

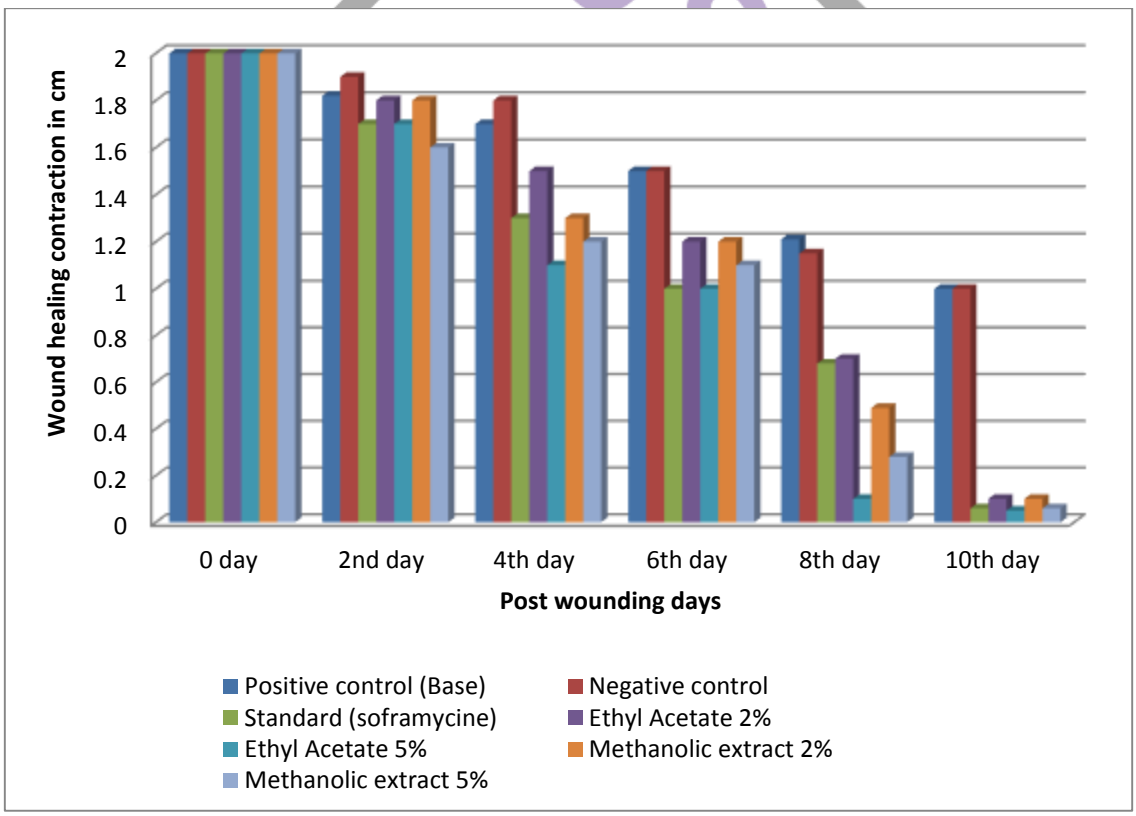



**Chart 3 : Comparative *In-vivo* wound healing activity of *Trigonella Foenum- graecum* leaves extracts**

**DISCUSSION:**

- No methodical reports on wound healing activity of *Trigonella Foenum-graecum* leaves are available.
- Preliminary phytochemical evaluation of both two extracts was carried out for the determination of presence of phytoconstituents. It reveals that all two extracts (i.e. ethyl acetate and methanol) contain carbohydrates, glycosides, coumarins, steroids, flavonoids, saponins and tannins. The total Phenolic and Flavonoid content were also determined. It was found that the ethyl acetate extract has more phenols and flavonoid as compared with the methanolic extract.
- The *in vitro* Antibacterial property of *Trigonella Foenum-graecum* leaves was carried out by using agar cup and plate method. In this method increase in zone of inhibition was calculated and compared with standard (*Streptomycin)*).
- Ethyl acetate extract having better antibacterial activity on *Baccilus substillus* than *Staphylococcus aureus* & *E.coli.* Methanol extract having better antibacterial activity against *Baccilus substillus* & *Staphylococcus aureus* than *E.coli.*

- Antifungal property of *Trigonella Foenum-graecum* leaves was carried out by using poison plate method. In this method, reducing growth of fungi (moderate antifungal activity) and no growth of fungi of test sample was calculated and compared with standard i.e (*Griseofulvin*). Both extract showed the reduced growth (more than 50% and less than 90% reduction in growth) at 100 mg/ml.
- The acute oral toxicity study was also determined according to OECD guidelines. After administration of 2000 mg/kg of dose animals does not showing any adverse reaction.
- *In-vivo* wound healing activity of ethyl acetate and methanol extract of *Trigonella Foenum-graecum* was evaluated by using the excision wound model. The test groups i.e. TFG-EA 2%, TFG-EA 5%, TFG-M 2% and TFG-M 5% showed highly significant decrease in wound area when compared with positive control group. The test groups i.e. TFG-EA 5% and TFG-M 5% showed highly significant decrease in wound area when compared with the standard except TFG-EA 2%, TFG-M 2% as it showed significant decrease in wound area.

**SUMMARY AND CONCLUSION:**

- ❖ *Trigonella Foenum-graecum Linn* (Fabaceae) is widely distributed in India. It is commonly known as Fenugreek. The fresh leaves of *Trigonella Foenum-graecum* were collected from local market of Nanded & authenticated from Yashwant Mahavidyalaya, Nanded.
- ❖ The collected dried leaves were properly pulverized & used for extraction. From the continuous hot extraction, using Soxhlet apparatus, three extracts namely Petroleum ether (60-80$^0$C), Ethyl acetate (77.1ºC) & Methanol (64.7ºC) were obtained.
- ❖ After subjecting to phytochemical screening of extracts showed the presence of carbohydrates, glycosides, flavonoids, tannins, coumarins, steroids, saponins & amino acids. The TLC finger printing of extracts using various solvent proportions showed different colored spots. The $R_f$ values of these spots were measured.
- ❖ The extracts were also studied to determine their total phenolic & flavonoid contents. FolinCiocaltue reagent method was used for total phenolic contents; the calibration curve was obtained from gallic acid in concentrations of (50, 100, 150, 200, 250 µg/ml) & the total phenolic content was expressed as gallic acid equivalent in mg/g of the extract. Aluminium trichloride complexetion method was used for determination of total flavonoid content, the calibration curve was drawn using various concentrations of rutin (100, 200, 300, 400, 500 µg/ml). The total flavonoid content was expressed as rutin equivalent in mg/g of the extract.
- ❖ The evaluation of antioxidant activity of *Trigonella Foenum-graecum* leaves extracts was done by using the DPPH scavenging activity at concentrations of 10, 20, 30, 40 & 50µg/ml. All the three extracts showed maximum antioxidant activity at 50µg/ml. In DPPH method, percentage scavenging activity as percent inhibition was calculated and compared with the standard (Ascorbic acid).
- ❖ The result of acute oral toxicity of plant extract as per standard references revealed that in single dose the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in rat. Accordingly safe dose was considered for experimental dose as ≤2000mg/kg.
- ❖ For studying *in-vivo* wound healing activity, 42 wistar rats were required. The animals were randomly divided into seven groups of six animals in each group namely:- Positive control, negative control, standard, Test dose 1 (2% TFG EA), Test dose 2 (5% TFG EA), Test dose 3 (2% TFG M) & Test dose 4 (5% TFG M). Animals housed in separate cages under control.
- ❖ The observation of % wound closure is done on 2$^{nd}$, 4$^{th}$, 6$^{th}$, 8$^{th}$ & 10$^{th}$ post wounding days. The ointment of plant extract, reference standard & simple ointment base is applied daily until the recovery.
- ❖ The test groups i.e. TFG-EA 2%, TFG-EA 5%, TFG-M 2% and TFG-M 5% showed highly significant decrease in wound area when compared with positive control group. The test groups i.e. TFG-EA 5% and TFG-M 5% showed highly significant decrease in wound area when compared with the standard except TFG-EA 2% and TFG-M 2%, as it showed significant decrease in wound area
- ❖ Similarly promising antibacterial & antifungal activity against selected bacterial & fungi species of plant extracts may therapeutically useful as it will protect infection in wound. Overall extracts of leaves of *Trigonella Foenum-graecum* showed high potential for its wound healing activity.

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