



## Formulation and Evaluation of Polyherbal Gel Comprising of Amla, Neem, Mulethi and Tulsi for Treatment of Canker Sores

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### Abstract:

Amla, Neem, Mulethi and Tulsi are known to have medicinal properties. These herbs are quite beneficial for treating many diseases. Amla, Neem, Mulethi and Tulsi have been used by individuals to cure respiratory tract infections, gastrointestinal conditions, cough, cold, bronchitis, and asthma. It has also been observed that the immunological condition has improved. These historic uses of plant medications include treating heart disease, hepatitis, fungal infections, malaria, psoriasis, ulcers, and sores. In the present research polyherbal gel formulation of Amla, Neem, Mulethi and Tulsi extracts for Mouth ulcer are prepared and evaluated. Drug compatibility with specific excipients used in the formulation was tested using densitometry TLC to determine the drug concentration. The almost similar R<sub>f</sub> values that were obtained indicated that Amla, Neem, Mulethi and Tulsi were compatible with the excipients utilized in the gel formulation. Percentage drug release study, thus conducted, revealed that about 81.110% Gallic acid (Amla), 82.006% Azadirachtine (Neem), 83.556%

Glycyrrhizic acid (Mulethi) and 81.744 % Ursolic acid (Tulsi) were released from the prepared polyherbal gel. Ex-vivo drug release revealed that about 83.301% Gallic acid (Amla), 86.594% Azadirachtine (Neem),

86.989% Glycyrrhizic acid (Mulethi) and 83.413% Ursolic acid (Tulsi) were released from the prepared polyherbal gel. In conclusion, polyherbal gel may be clinically effective in treating canker sores. It may also be worth exploring further for other ulcerative disorders with topical or surface origins.

**Keywords:** Polyherbal, gel, amla, neem, mulethi, tulsi, canker sores, gallic acid, azadirachtine, glycyrrhizic acid, ursolic acid

## 1. Introduction

Aphthous ulcers, another name for canker sores, are tiny, excruciating lesions that develop on the soft tissues of the mouth or at the gum base. It may be quite uncomfortable for the person with these sores to eat, drink, or speak. While canker sores are usually painless and go away on their own in a week or two, the discomfort and inconvenience they cause can have a serious negative effect on a person's quality of life. Although the precise etiology of canker sores is still unknown, a number of things have been linked to their development, including minor accidents, dietary allergies, stress, hormonal changes, and underlying health conditions [1-4].

Oral drugs, mouth rinses, and topical ointments are some of the therapies available for canker sores in both conventional and modern medicine. These therapies might not be appropriate for everyone, though, and they frequently have negative consequences. Consequently, there has been an increase in demand for safe, natural remedies that are holistic and effective. Herbal medicines are among the most popular because of their strong therapeutic effects and low adverse effects. The utilization of numerous herbs in polyherbal compositions has demonstrated significant potential in augmenting overall efficacy via synergistic effects. This method works especially well for treating complicated disorders like canker sores, which have a number of underlying causes, including inflammation, microbial infection, and inadequate healing [5-7].

Amla (Indian Gooseberry), Neem, Mulethi (Licorice) and Tulsi (Holy Basil) herbs has a rich history in traditional medicine and is well-documented for its medicinal properties. Combining these herbs into a single gel formulation aims to provide a comprehensive treatment that addresses the various aspects of canker sores, from pain relief to accelerated healing and prevention of recurrence. By combining these herbs into a single gel composition, the goal is to offer a whole therapy that takes care of all the many elements of canker sores, from pain management to faster healing and recurrence prevention [8-12].

Amla is well known for having a high vitamin C content and strong antioxidant qualities; it also aids in tissue healing and inflammation reduction. Amla's antioxidant activities help shield the oral tissues from more damage, and its anti-inflammatory qualities can help reduce the discomfort and swelling caused by canker sores. Neem helps to manage infections and reduce inflammation because of its well-known antibacterial and anti-inflammatory properties. With its proven antibacterial, antifungal, and antiviral qualities, neem is useful in avoiding secondary infections that worsen canker sores. This is because it includes substances like nimbin and nimbidin [13-16].

Mulethi is well-known for having antibacterial and anti-inflammatory qualities; it helps to lessen pain and hasten the healing process. It has been shown that the glycyrrhizin contained in mulethi has calming effects on mucous membranes, which helps to lessen canker sore pain and irritation. Tulsi improves the body's innate healing mechanisms and is well-known for its immunomodulatory, anti-inflammatory, and antibacterial properties. A wonderful addition to the polyherbal gel, tulsi contains essential oils and bioactive chemicals like eugenol that have been demonstrated to decrease inflammation and improve healing [17-20].

Direct treatment at the ulcer site is provided by the combination of these herbs in a gel form, which is a useful and effective topical use. In addition to treating the symptoms, this

polyherbal gel tackles the underlying causes of canker sores, accelerating healing and avoiding recurrence. The subsequent sections will delve into the formulation process and evaluation parameter of formulated polyherbal gel. This investigation will shed light on how these herbal components synergistic actions may be able to give a safe and efficient remedy for people experiencing canker sore discomfort [21].

## 2. Materials and Methods

### 2.1 Materials

All of the chemicals and reagents utilized in the study, including solvents and other materials needed to prepare the formulation, were purchased from several well-known companies.

### 2.2 Adopted Method

#### 2.2.1 Pharmacognostical Studies

##### 2.2.1.1 Collection of the plant material

The plant material (Amla, Neem, Mulethi and Tulsi) was collected from the local market.

##### 2.2.1.2 Extraction

Following a thorough washing in distilled water, each plant material was dried at 60-70°C, ground into a coarse powder, and then placed in a separate, clean container. A predetermined quantity of every crushed material was immersed in an adequate amount of distilled water, allowed to stand overnight, and then filtered. A lyophilizer was used to collect and freeze-dry each individual filtrate at -60°C. For use in additional research, each lyophilized extract was kept in an airtight container [22].

#### 2.2.2 Phytochemical Studies

##### 2.2.2.1 Phytochemical screening

A range of phytochemical analyses were performed utilizing each powdered extract of selected drugs [23].

**2.2.2.1.1 Test for alkaloids:** Included different tests i.e; Mayer's, Hagger's, Wagner's and Dragendroff's tests, according to official procedures.

**2.2.2.1.2 Test for flavonoids:** Included a lead acetate and NaOH test that was carried out using the prescribed procedures for each extract.

**2.2.2.1.3 Test for Phenolics and Tannins:** Included 5% ferric chloride solution, Lead acetate, Gelatin tests, Bromine water, Acetic acid solution, Potassium di- chromate, Dil HNO<sub>3</sub> and Dil NH<sub>4</sub>OH + potassium ferricyanide solution in accordance with the official descriptions.

#### 2.2.3 Drug Compatibility Studies

Using a TLC examination, the compatibility of the generated aqueous extracts with each plant material was assessed [24].

##### 2.2.3.1 Thin layer chromatographic studies

To perform thin layer chromatography, materials were dissolved in distilled water. The extract was spotted on pre-coated TLC plates composed of the stationary phase, silica gel GF254, and the mobile phases were created especially for each herbal extract, which were as follows: A blend of toluene ethyl acetate Amla was treated with formic acid (60: 30: 10), whereas neem was treated with ethyl acetate and ethanol (1:3). Mulethi was mixed with a mixture of acetone and regular hexane (3:10 v/v), whereas Tulsi was mixed with a mixture of methanol and chloroform (95:5). Each extract's R<sub>f</sub> value was calculated and noted, for each extract alone and with other ingredients. Table 1 summarizes the composition of polyherbal gel.

#### 2.2.4 Formulation of Polyherbal Gel

**Table 1.** Composition of polyherbal gel

| S No. | Ingredients | Quantity |
|-------|-------------|----------|
| 1.    | Amla        | 1gm      |
| 2.    | Neem        | 1gm      |

|    |                                       |       |
|----|---------------------------------------|-------|
| 3. | Mulethi                               | 1gm   |
| 4. | Tulsi                                 | 1gm   |
| 5. | Carbopol 934(gelling agent)           | 3gm   |
| 6. | Polyethylene glycol 400 (plasticizer) | 1 gm  |
| 7. | Aspartame (sweetener)                 | 1%    |
| 8. | Sodium benzoate (preservative)        | 1%    |
| 9. | Distilled water                       | 100gm |

With constant heating and mechanical stirring, 3 grams of precisely weighed carbopol was dissolved in 100ml of purified water. A pre-measured amount of polyethylene glycol 400 was diluted. Aspartame and sodium benzoate were added in precisely measured amounts after the solution had cooled.

Finally, required quantity of each dried extracts (Amla, Neem, Mulethi and Tulsi), was mixed to above mixture. After the material was thoroughly homogenized in the homogenizer, sodium hydroxide solution was used to adjust the pH to 6.8.

### 3. Evaluation Parameter of Topical Gel Formulations

#### 3.1 Clarity

The prepared gel was seen against a dark and white background to ensure that it was clear.

#### 3.2 pH

A digital pH meter was used to measure the pH of the gel compositions. After precisely weighing 2.5g of gel, it was mixed with 25ml of purified water and kept for two hours. The formulation's pH was measured three times.

#### 3.3 Viscosity

The Brookfield viscometer was used to measure the gel's viscosity. Using a Brookfield viscometer, the rheological properties of gel were examined at 25°C. Using spindle number 60, the measurement was taken at 100 rpm for 30 seconds.

#### 3.4 Spreadability

The word "spreadability" refers to the amount of skin that the gel easily spreads over after being applied or that is totally influenced by each other.

$$S = ml/t$$

Where,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken to separate the slide

#### 3.5 In vitro diffusion study

The release of active ingredients from various medications included in Polyherbal gel was investigated through the use of an open-ended diffusion cell and a dialysis membrane. After being pre-soaked in phosphate buffer (pH 6.8), a dialysis (cellophane) membrane was positioned between the donor and the receptor compartment. In the permeation cell, 1gm of medication was stored. 200 ml of pH 6.8 buffer was kept in the receptor compartment and continuously agitated to maintain a temperature of 37°C ± 5°C. Every so often, an aliquot of 5 ml was removed and replaced with new medium. After the proper dilutions, collected samples were subjected to HPLC analysis.

#### 3.6 Ex- vivo study

Ex vivo study was performed using Franz diffusion cell. A dialysis (Buccal membrane (Goat)) membrane was pre-soaked in phosphate buffer (pH 6.8) and mounted between the donor and the receptor compartments. About 1 gm formulation (polyherbal gel) was kept on the membrane. The receptor compartment contained 30 ml of pH 6.8 phosphate buffer and was constantly stirred at temperature 37 ± 5°C Aliquots (5ml) were withdrawn periodically and analyzed HPLC after suitable dilutions[25, 26].

#### 4. Result and Discussion

##### 4.1 Phytochemical Studies

##### 4.1.1 Phytochemical screening

**Table 2:** Phytochemical analyses to identify the organic components found in Amla (*Embolica officinalis*)

| Chemical class      | Tests               | Inference  |   |
|---------------------|---------------------|--|---|
| Alkaloids           | Mayer's test        | +  |   |
|                     | Wagner's test       | +  |   |
|                     | Hager's test        | -  |   |
|                     | Dragenderoff's test | +  |   |
| Flavonoids          | Lead acetate test   | +  |   |
|                     | NaOH test           | +  |   |
| Phenolic<br>Tannins | and                 | 5% FeCl <sub>3</sub> solution                            | - |
|                     |                     | Lead acetate   | + |
|                     |                     | Gelatine solution  | + |
|                     |                     | Bromine water  | + |
|                     |                     | Acetic acid solution                                     | - |
|                     |                     | Potassium di chromate                                    | + |
|                     |                     | Dil HNO <sub>3</sub>                                     | + |
|                     |                     | Dil NH <sub>4</sub> OH + Potassium ferricyanide solution | + |

**Table 3:** Phytochemical tests for detection of organic constituents present in Neem(*Azadirachta indica*)

| Chemical class      | Tests               | Inference  |   |
|---------------------|---------------------|--|---|
| Alkaloids           | Mayer's test        | +  |   |
|                     | Wagner's test       | +  |   |
|                     | Hager's test        | +  |   |
|                     | Dragenderoff's test | +  |   |
| Flavonoids          | Lead acetate test   | +  |   |
|                     | NaOH test           | +  |   |
| Phenolic<br>Tannins | and                 | 5% FeCl <sub>3</sub> solution                            | - |
|                     |                     | Lead acetate   | + |
|                     |                     | Gelatine solution  | + |
|                     |                     | Bromine water  | + |
|                     |                     | Acetic acid solution                                     | + |
|                     |                     | Potassium di chromate                                    | + |
|                     |                     | Dil HNO <sub>3</sub>                                     | + |
|                     |                     | Dil NH <sub>4</sub> OH + Potassium ferricyanide solution | + |

**Table 4:** Phytochemical tests for detection of organic constituents present in Mulethi (*Glycyrrhiza glabra*)

| Chemical class | Tests               | Inference |
|----------------|---------------------|-----------|
| Alkaloids      | Mayer's test        | +         |
|                | Wagner's test       | +         |
|                | Hager's test        | -         |
|                | Dragenderoff's test | +         |
| Flavonoids     | Lead acetate test   | +         |

|                             |  |   |
|-----------------------------|--|---|
|                             | NaOH test  | + |
| <b>Phenolic Tannins and</b> | 5% FeCl <sub>3</sub> solution                            | + |
|                             | Lead acetate   | - |
|                             | Gelatine solution  | + |
|                             | Bromine water  | + |
|                             | Acetic acid solution                                     | + |
|                             | Potassium di chromate                                    | - |
|                             | Dil HNO <sub>3</sub>                                     | + |
|                             | Dil NH <sub>4</sub> OH + Potassium ferricyanide solution | + |

**Table 5:** Phytochemical tests for detection of organic constituents present in Tulsi (*Ocimum sanctum*)

| Chemical class              | Tests  | Inference |
|-----------------------------|--|-----------|
| <b>Alkaloids</b>            | Mayer's test   | +         |
|                             | Wagner's test  | -         |
|                             | Hager's test   | +         |
|                             | Dragenderoff's test                                      | +         |
| <b>Flavonoids</b>           | Lead acetate test  | +         |
|                             | NaOH test  | +         |
| <b>Phenolic Tannins and</b> | 5% FeCl <sub>3</sub> solution                            | +         |
|                             | Lead acetate   | +         |
|                             | Gelatine solution  | +         |
|                             | Bromine water  | +         |
|                             | Acetic acid solution                                     | +         |
|                             | Potassium di chromate                                    | -         |
|                             | Dil HNO <sub>3</sub>                                     | +         |
|                             | Dil NH <sub>4</sub> OH + Potassium ferricyanide solution | +         |

## 4.2 Drug Compatibility Studies

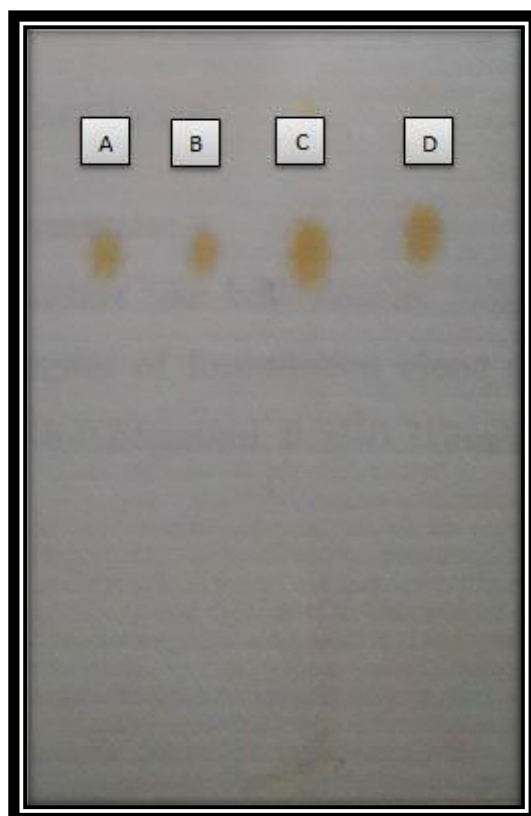
### 4.2.1 Thin Layer Chromatography

#### 4.2.1.1 Amla (*Emblica officinalis*)

The R<sub>f</sub> value of Amla (*Emblica officinalis*) with different excipients was found to be almost identical to that of the pure extract, indicating that the excipients were compatible with one another.

**Table 6:** Thin Layer Chromatography of Amla

| Spot No. | Ingredients       | R <sub>f</sub> value |
|----------|-------------------|----------------------|
| A        | Amla              | 0.75                 |
| B        | Amla+ Carbopol    | 0.74                 |
| C        | Amla + PEG        | 0.77                 |
| D        | Formulation blend | 0.81                 |



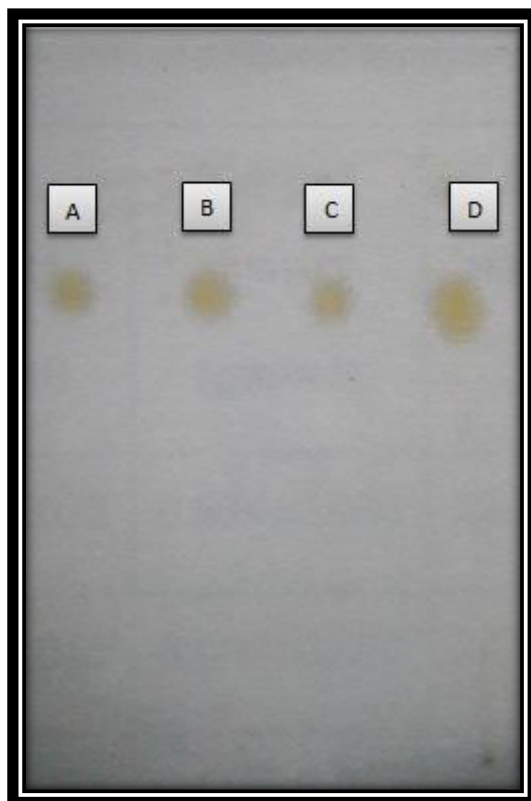
**Figure 1:** Photographic image of TLC of Amla (*Emblica officinalis*)

#### 4.2.1.2 Neem (*Azadirachta indica*)

Neem (*Azadirachta indica*) Rf value with several excipients was found to be almost identical to that of the pure extract, indicating that it was compatible with other substances.

**Table 7:** Thin Layer Chromatography of Neem

| Spot No. | Ingredients       | Rf value |
|----------|-------------------|----------|
| A        | Neem              | 0.65     |
| B        | Neem + Carbopol   | 0.70     |
| C        | Neem+ PEG         | 0.68     |
| D        | Formulation blend | 0.72     |



**Figure 2:** Photographic images of Thin Layer Chromatography of Neem (*Azadirachta indica*)

#### 4.2.1.3 Mulethi (*Glycyrrhiza glabra*)

Mulethi (*Glycyrrhiza glabra*) Rf values with different excipients were found to be almost identical to those of the pure extract; as a result, both were proven to be compatible with one another.

**Table 8:** Thin Layer Chromatography of Mulethi

| Spot No. | Ingredients        | Rf value |
|----------|--------------------|----------|
| A        | Mulethi (extract)  | 0.76     |
| B        | Mulethi + Carbopol | 0.78     |
| C        | Mulethi + PEG      | 0.81     |
| D        | Formulation blend  | 0.84     |





**Figure 3:** Photography image of Thin layer chromatography of Mulethi (*Glycyrrhiza glabra*)  
**4.2.1.4 Tulsi (*Ocimum sanctum*)**

As a result, both the pure extract and the Rf value of tulsi (*Ocimum sanctum*) with different excipients were found to be substantially identical, making them compatible.

**Table 9:** Thin Layer Chromatography of Tulsi

| Spot No. | Ingredients       | Rf value |
|----------|-------------------|----------|
| A        | Tulsi (extract)   | 0.59     |
| B        | Tulsi + Carbopol  | 0.55     |
| C        | Tulsi + PEG       | 0.58     |
| D        | Formulation blend | 0.63     |



**Figure 4:** Photographic image of Thin Layer Chromatography of Tulsi (*Ocimum sanctum*)

#### 4.3 Evaluation Parameters of Polyherbal gel

##### 4.3.1 Evaluation of polyherbal gel formulation

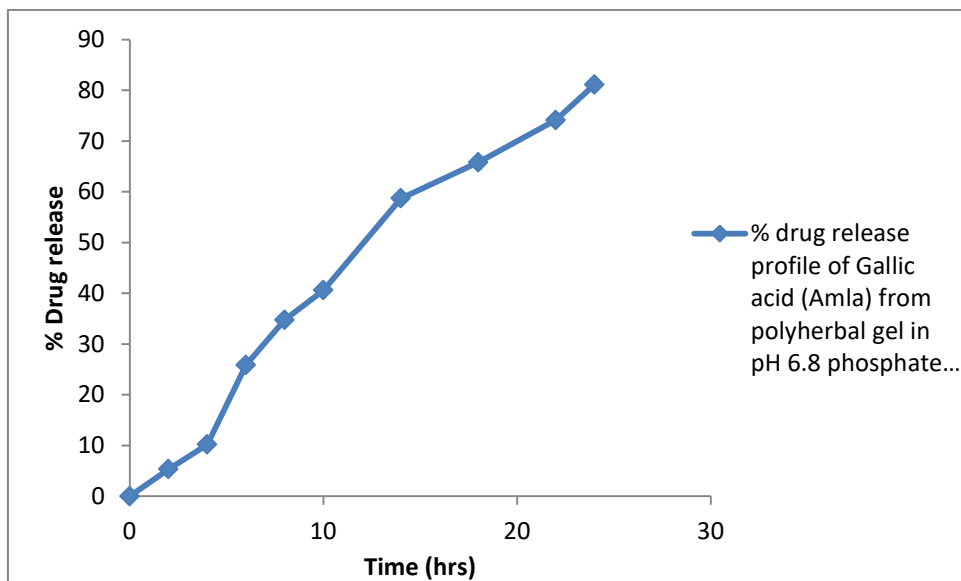
Polyherbal gel thus prepared was subjected to various evaluation parameters such as; clarity, pH, spreadability, viscosity and in-vitro diffusion study which revealed that prepared formulation showed good results and considered as best gel.

- Clarity:** Prepared gel was clear.
- pH:** pH of the formulation was found to be 6.8 which was similar to salivary pH.
- Viscosity:** Viscosity of gel was found to be 6870 cps.
- Spreadability:** Spreadability was found to be 9.6 gm.cm/sec.
- In-vitro diffusion study:** Percentage drug release from polyherbal gel formulation was found to be 81.110% Gallic acid (Amla), 82.006% Azadirachtine (Neem), 83.556% Glycyrrhizic acid (Mulethi), and 81.744% Ursolic acid (Tulsi) in pH 6.8 phosphate buffer within 24 hrs.

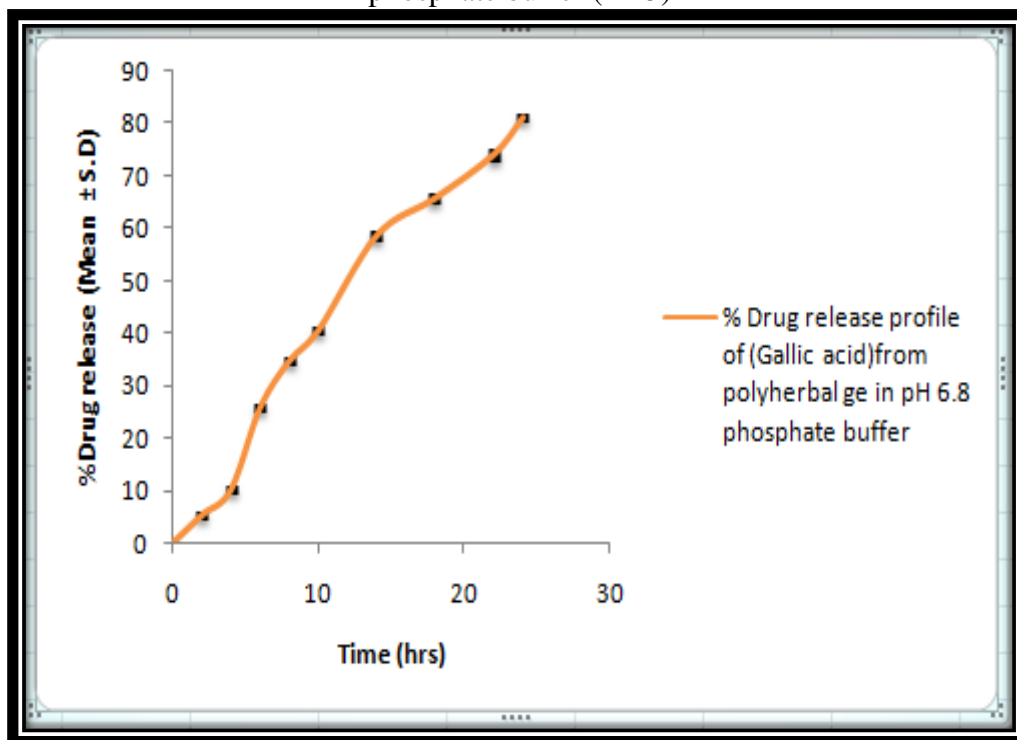
##### 4.3.2 Percentage drug release (in-vitro diffusion study)

**Table 10:** Percentage drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer (Mean  $\pm$  S.D)

| S. No. | Time(hrs) | Percentage drug release (Mean $\pm$ S.D) |
|--------|-----------|--|
| 1.     | 0         | 0  |
| 2.     | 2         | 5.320 $\pm$ 0.251                        |
| 3.     | 4         | 10.191 $\pm$ 0.241                       |
| 4.     | 6         | 25.848 $\pm$ 0.248                       |
| 5.     | 8         | 34.743 $\pm$ 0.245                       |
| 6.     | 10        | 42.5 $\pm$ 0.254                         |
| 7.     | 14        | 57.904 $\pm$ 0.419                       |
| 8.     | 18        | 64.643 $\pm$ 0.265                       |
| 9.     | 22        | 73.102 $\pm$ 0.520                       |
| 10.    | 24        | 81.110 $\pm$ 0.159                       |



**Figure 5:** Percentage drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer (n= 3)

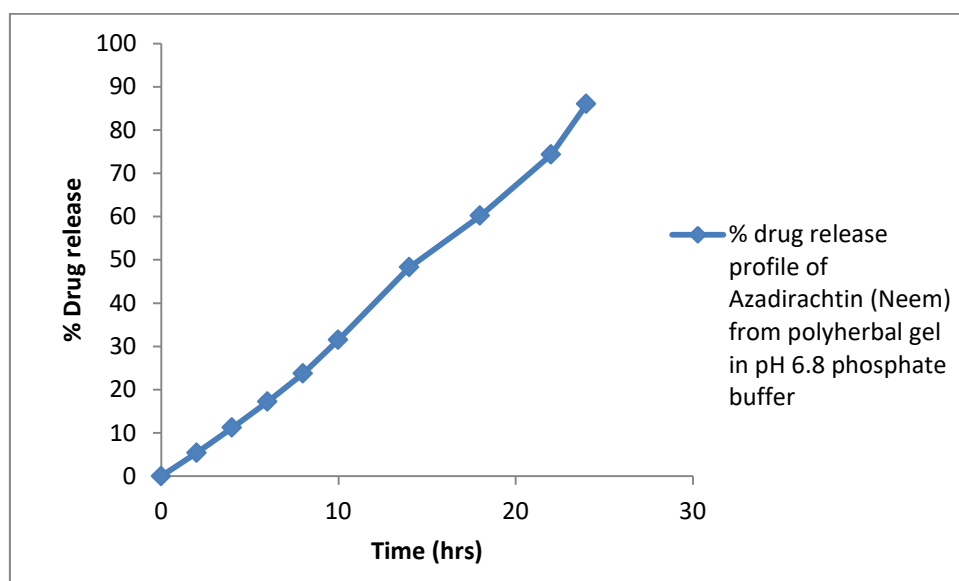


**Figure 6:** Percentage drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer (Mean ± S.D)

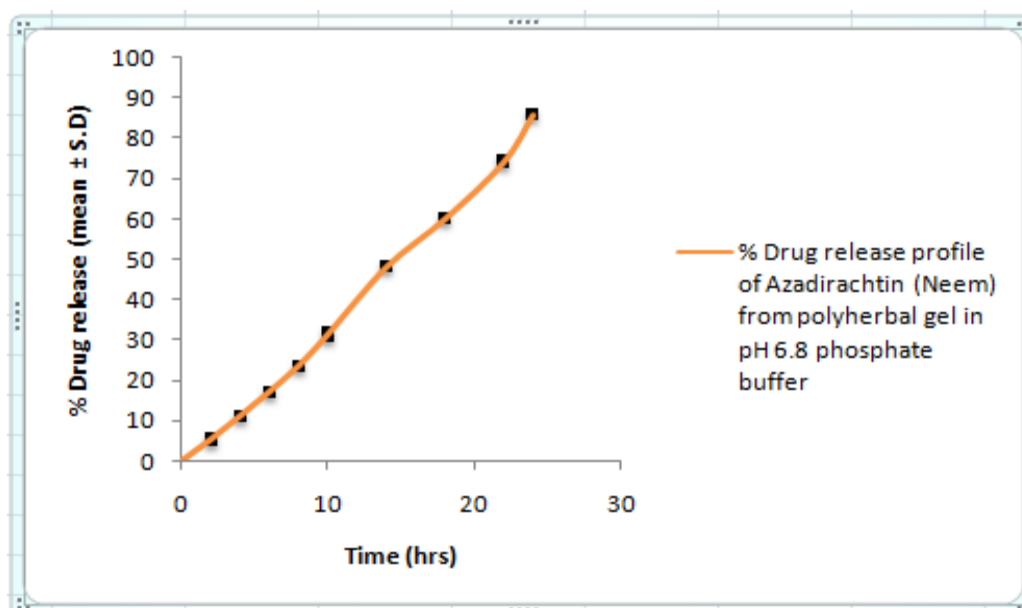
**Table 11:** Percentage drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer (Mean ± S.D)

| S. No. | Time (hrs) | Percentage drug release (mean ± S.D) |
|--------|------------|--------------------------------------|
| 1.     | 0          | 0                                    |
| 2.     | 2          | 5.378 ± 0.056                        |
| 3.     | 4          | 12.209 ± 0.467                       |
| 4.     | 6          | 17.208 ± 0.559                       |
| 5.     | 8          | 24.695 ± 0.472                       |
| 6.     | 10         | 31.527 ± 0.886                       |

|     |    |                |
|-----|----|----------------|
| 7.  | 14 | 49.259 ± 0.474 |
| 8.  | 18 | 60.190 ± 0.510 |
| 9.  | 22 | 76.308 ± 0.801 |
| 10. | 24 | 82.006 ± 0.462 |



**Figure 7:** Percentage drug release profile of Azadirachtin (Neem) from polyherbal gel in pH 6.8 phosphate buffer (n=3)

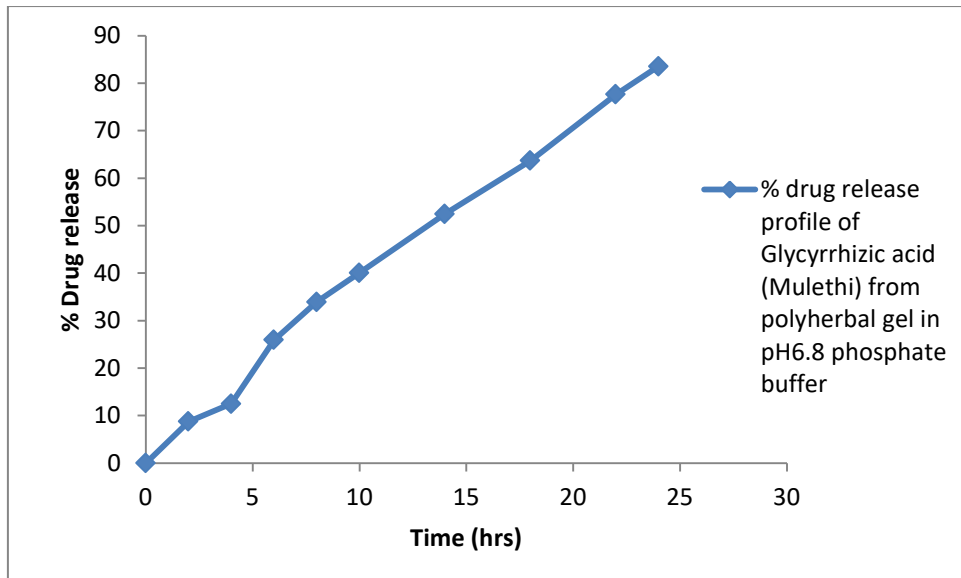


**Figure 8:** Percentage drug release profile of Azadirachtin (Neem) from polyherbal gel in pH 6.8 phosphate buffer (Mean ± S.D)

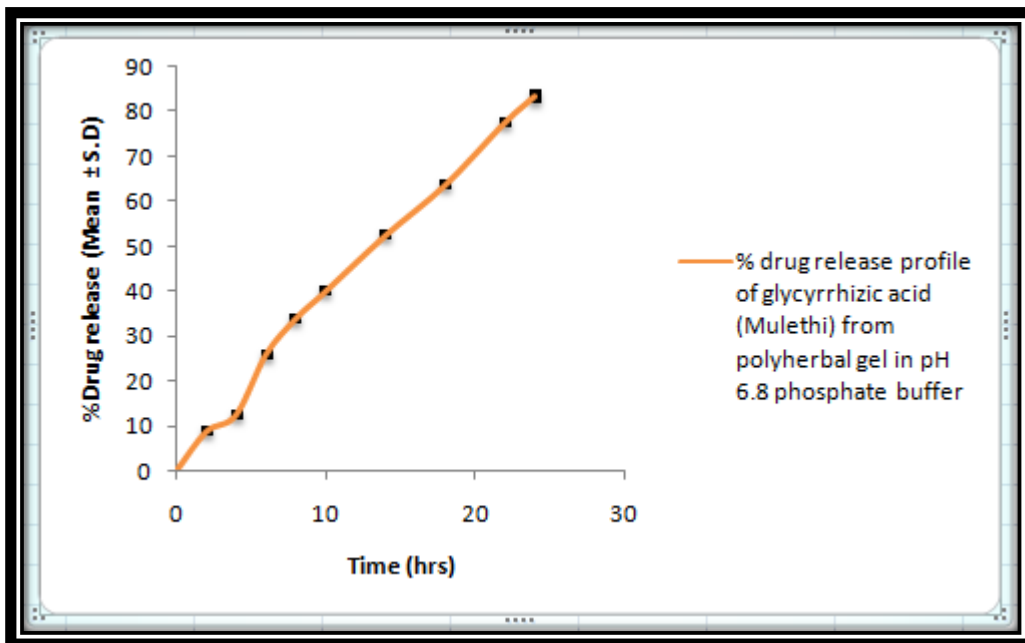
**Table 12:** Percentage drug release of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer (Mean ± S.D)

| S. No. | Time (hrs) | Percentage drug release (Mean ± S.D) |
|--------|------------|--------------------------------------|
| 1      | 0          | 0                                    |
| 2.     | 2          | 9.760 ± 0.208                        |
| 3.     | 4          | 12.485 ± 0.411                       |
| 4.     | 6          | 26.942 ± 0.231                       |

|     |    |                |
|-----|----|----------------|
| 5.  | 8  | 34.936 ± 0.374 |
| 6.  | 10 | 40.049 ± 0.302 |
| 7.  | 14 | 53.461 ± 0.157 |
| 8.  | 18 | 63.695 ± 0.168 |
| 9.  | 22 | 78.643 ± 0.155 |
| 10. | 24 | 83.556 ± 0.663 |



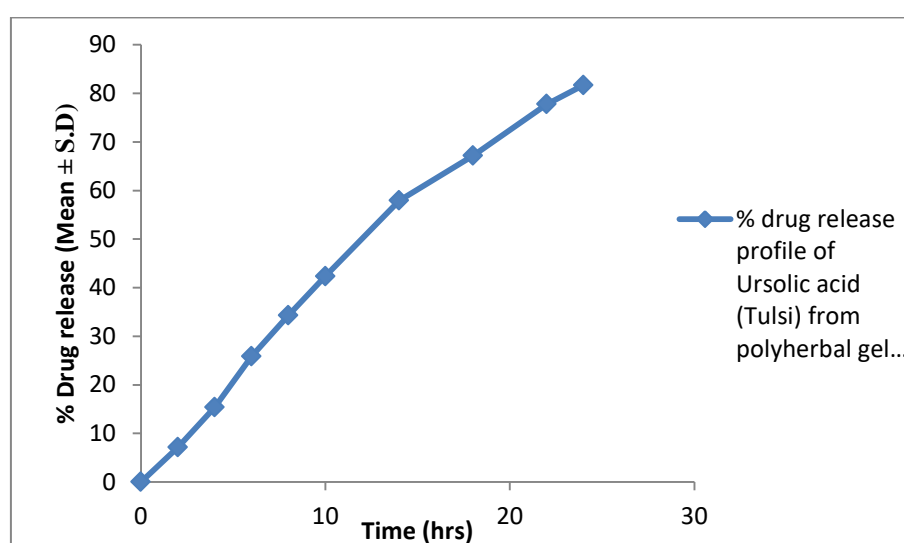
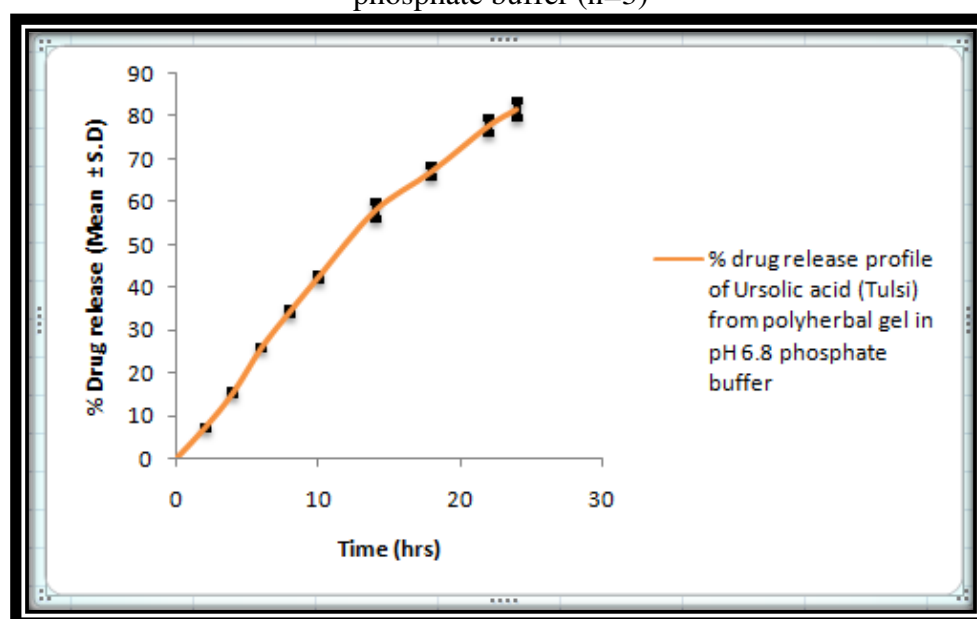
**Figure 9:** Percentage drug release of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer (n=3)



**Figure 10:** Percentage drug release of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer (Mean ± S.D)

**Table 13:** Percentage drug release of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer (Mean  $\pm$  S.D)

| S. No. | Time (hrs) | Percentage drug release (mean $\pm$ S.D) |
|--------|------------|--|
| 1.     | 0          | 0  |
| 2.     | 2          | 8.17 $\pm$ 0.151                         |
| 3.     | 4          | 16.361 $\pm$ 0.234                       |
| 4.     | 6          | 25.879 $\pm$ 0.247                       |
| 5.     | 8          | 35.310 $\pm$ 0.552                       |
| 6.     | 10         | 42.358 $\pm$ 0.722                       |
| 7.     | 14         | 56.949 $\pm$ 1.855                       |
| 8.     | 18         | 67.189 $\pm$ 1.528                       |
| 9.     | 22         | 78.773 $\pm$ 1.819                       |
| 10.    | 24         | 81.744 $\pm$ 1.898                       |

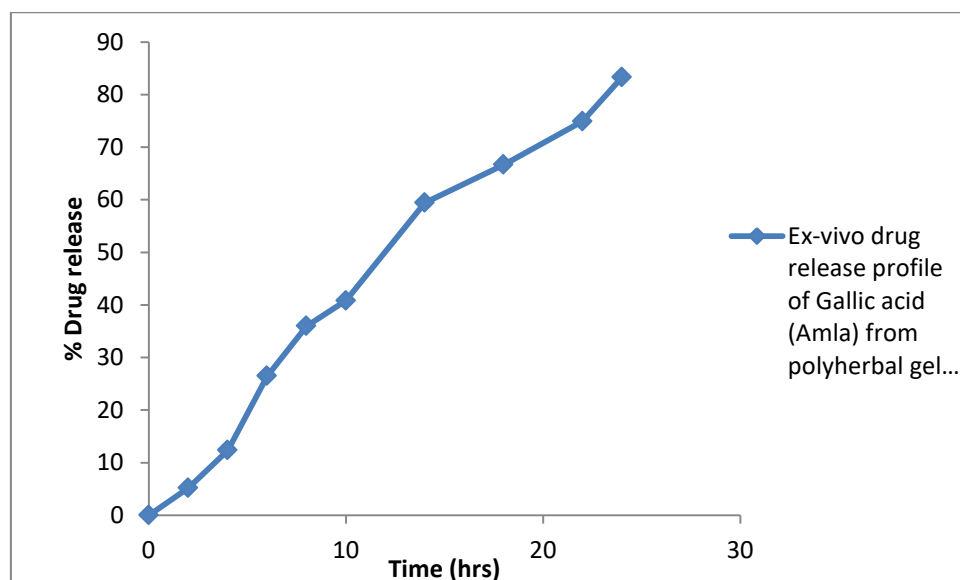
**Figure 11:** Percentage drug release of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer (n=3)**Figure 12:** Percentage drug release profile of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer (Mean  $\pm$  S.D)

### 4.3.3 Ex-vivo study of polyherbal gel

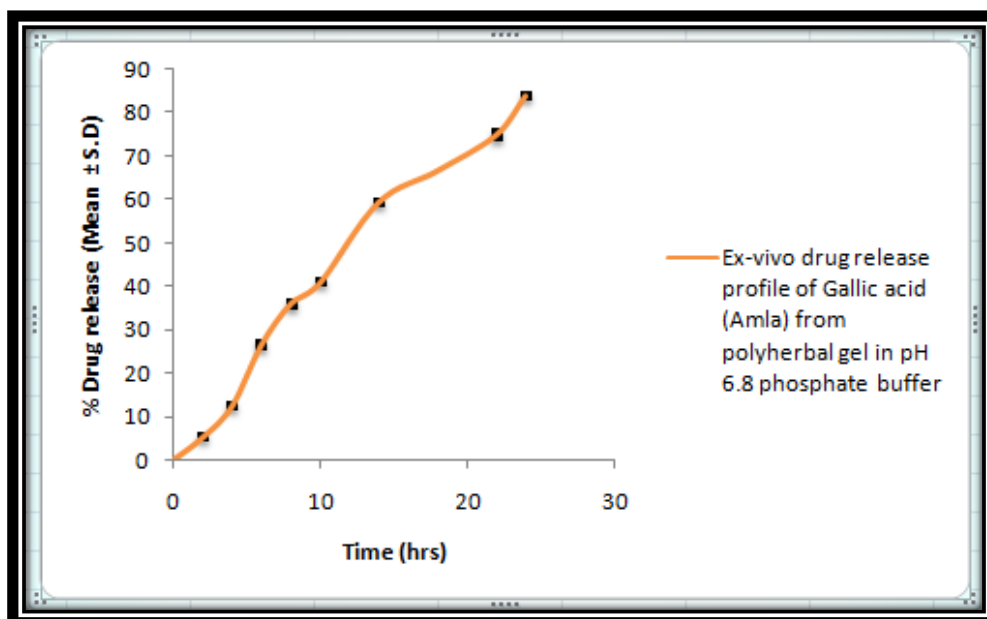
Ex-vivo drug release data of polyherbal gel was also obtained using a buccal membrane.

**Table 14:** Ex-vivo drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer

| S. No. | Time (hrs) | Percentage drug release (mean $\pm$ S.D) |
|--------|------------|--|
| 1.     | 0          | 0  |
| 2.     | 2          | 5.188 $\pm$ 0.251                        |
| 3.     | 4          | 13.375 $\pm$ 0.257                       |
| 4.     | 6          | 27.485 $\pm$ 0.258                       |
| 5.     | 8          | 35.975 $\pm$ 0.484                       |
| 6.     | 10         | 41.840 $\pm$ 0.252                       |
| 7.     | 14         | 59.425 $\pm$ 0.251                       |
| 8.     | 18         | 67.657 $\pm$ 0.135                       |
| 9.     | 22         | 74.925 $\pm$ 0.545                       |
| 10.    | 24         | 83.301 $\pm$ 0.328                       |



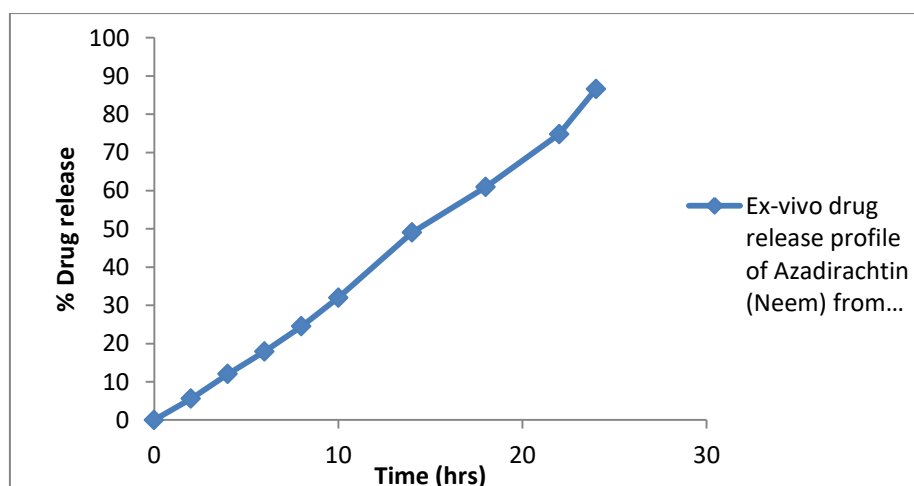
**Figure 13:** Ex-vivo drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (n=3)



**Figure 14:** Ex-vivo drug release profile of Gallic acid (Amla) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (Mean ± S.D)

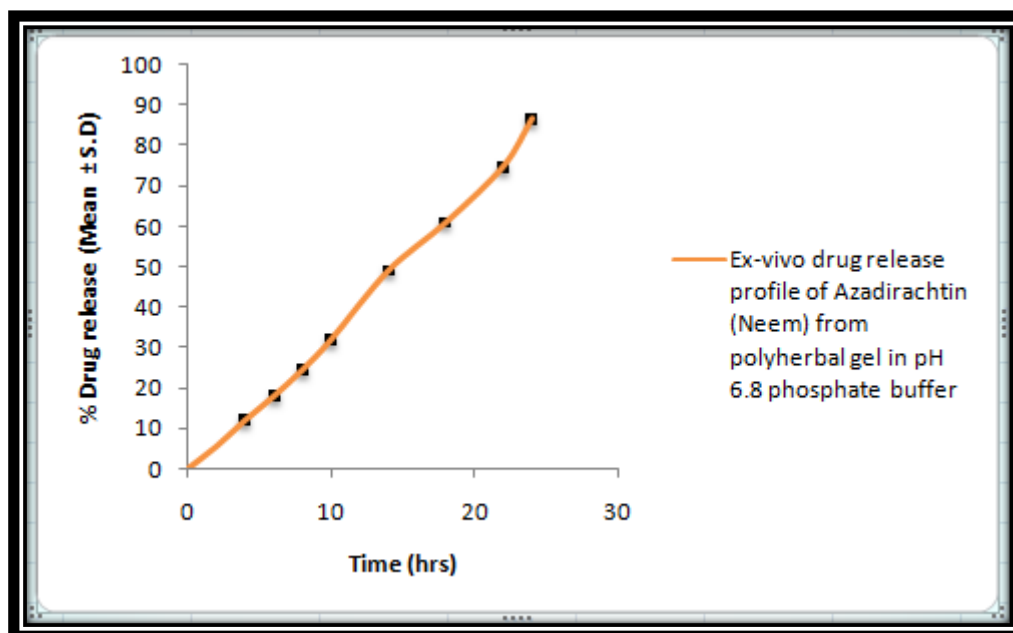
**Table 15:** Ex-vivo drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer

| S. No | Time | Percentage drug release (mean ± S.D) |
|-------|------|--------------------------------------|
| 1.    | 0    | 0                                    |
| 2.    | 2    | 7.59 ± 0.147                         |
| 3.    | 4    | 15.027 ± 0.478                       |
| 4.    | 6    | 19.963 ± 0.511                       |
| 5.    | 8    | 25.494 ± 0.478                       |
| 6.    | 10   | 35.009 ± 0.475                       |
| 7.    | 14   | 51.069 ± 0.477                       |
| 8.    | 18   | 63.955 ± 0.469                       |
| 9.    | 22   | 75.825 ± 0.465                       |
| 10.   | 24   | 86.594 ± 0.616                       |



**Figure 15:** Ex-vivo drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (n=3)

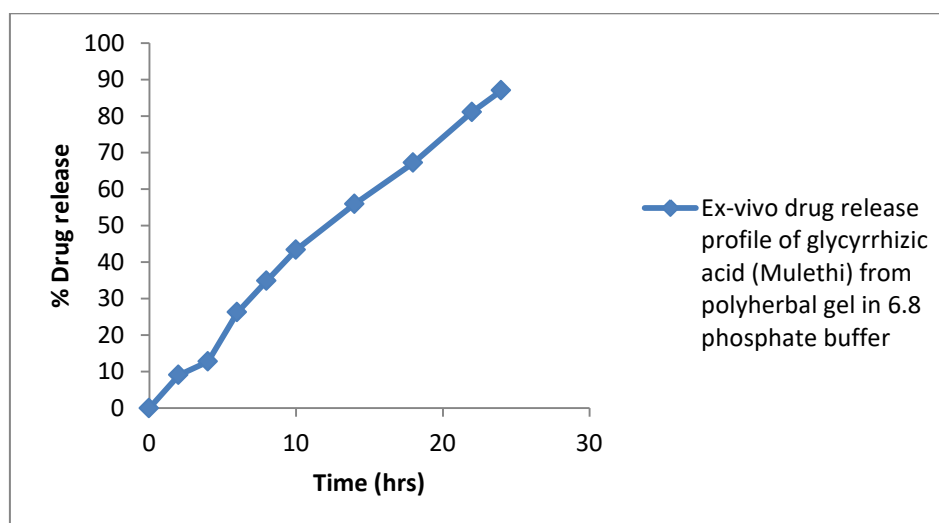




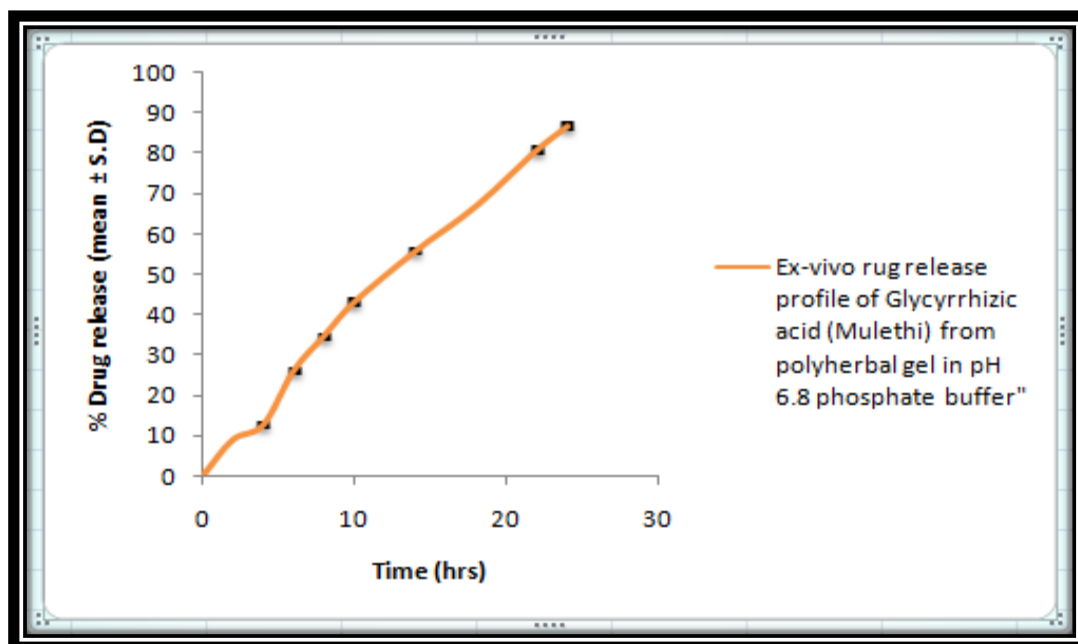
**Figure 16:** Ex-vivo drug release profile of Azadirachtin (Neem) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (Mean ± S.D)

**Table 16:** Ex-vivo drug release profile of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer

| S. No. | Time (hrs) | Percentage drug release (mean ± S.D) |
|--------|------------|--------------------------------------|
| 1.     | 0          | 0                                    |
| 2.     | 2          | 9.092 ± 0.211                        |
| 3.     | 4          | 12.789 ± 0.294                       |
| 4.     | 6          | 27.253 ± 0.211                       |
| 5.     | 8          | 36.88 ± 0.257                        |
| 6.     | 10         | 42.354 ± 0.278                       |
| 7.     | 14         | 53.939 ± 0.287                       |
| 8.     | 18         | 64.170 ± 0.225                       |
| 9.     | 22         | 79.095 ± 0.461                       |
| 10.    | 24         | 86.989 ± 0.677                       |



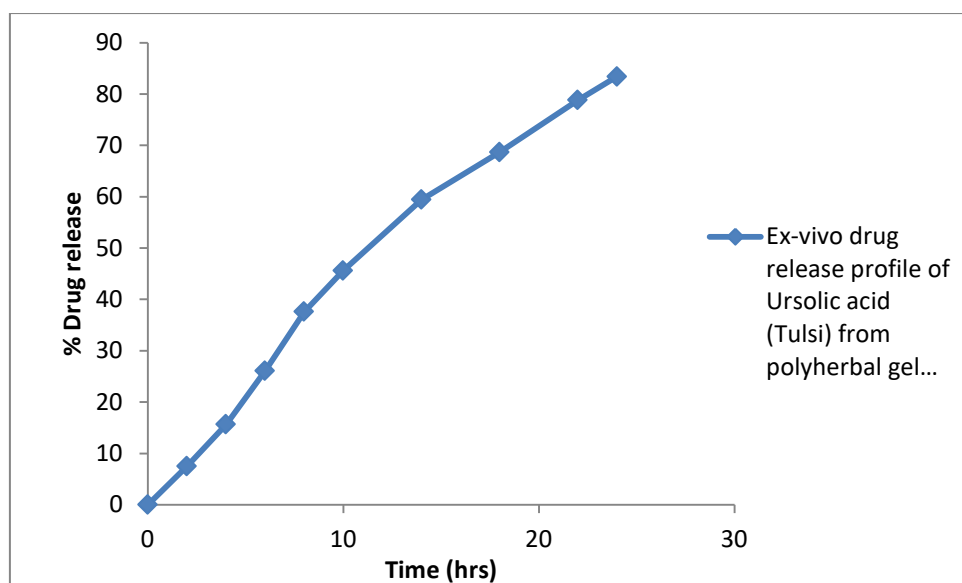
**Figure 17:** Ex-vivo drug release profile of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (n=3)



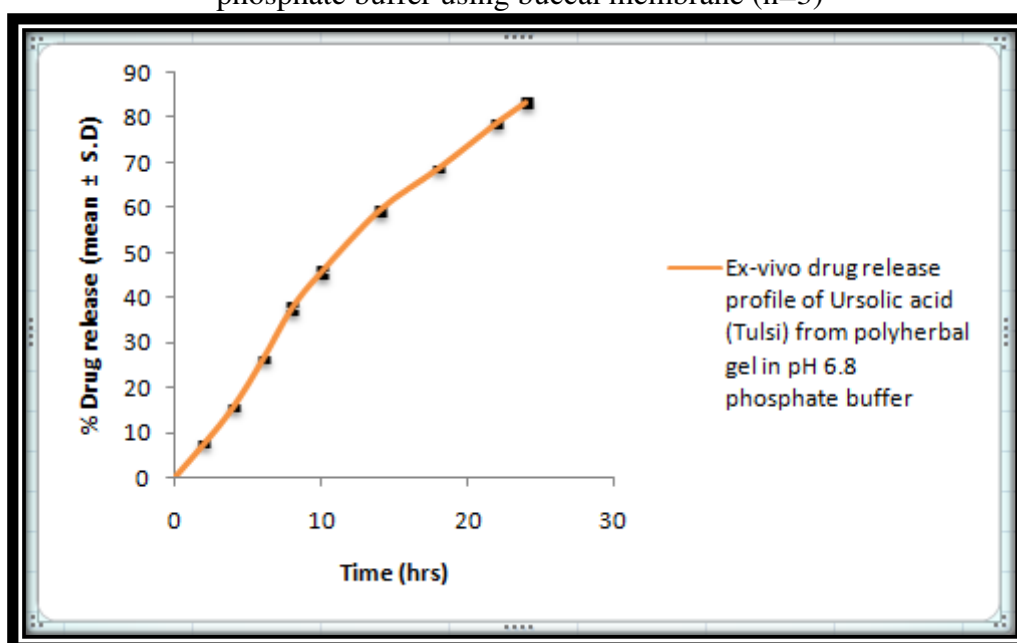
**Figure 18:** Ex-vivo drug release profile of Glycyrrhizic acid (Mulethi) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (Mean ± S.D)

**Table 17:** Ex-vivo drug release profile of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer

| S. No | Time (hrs) | Percentage drug release (mean ± S.D) |
|-------|------------|--------------------------------------|
| 1.    | 0          | 0                                    |
| 2.    | 2          | 8.501 ± 0.164                        |
| 3.    | 4          | 17.655 ± 0.222                       |
| 4.    | 6          | 27.119 ± 0.211                       |
| 5.    | 8          | 34.614 ± 0.994                       |
| 6.    | 10         | 42.605 ± 0.863                       |
| 7.    | 14         | 56.438 ± 0.745                       |
| 8.    | 18         | 65.694 ± 0.420                       |
| 9.    | 22         | 79.823 ± 0.523                       |
| 10.   | 24         | 83.413 ± 0.583                       |



**Figure 19:** Ex-vivo drug release profile of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer using buccal membrane (n=3)



**Figure 20:** Ex-vivo drug release profile of Ursolic acid (Tulsi) from polyherbal gel in pH 6.8 phosphate buffer in buccal membrane (Mean  $\pm$  S.D)

## 5. Conclusion

The present work carried out the research work aiming at increasing the residence time of drug at the site of application and preventing severe side effects associated with its topical administration. Considering the local healing the researchers designed an inexpensive formulation for the treatment of mouth ulcers as herbal formulations have a growing demand in the world market. In the present work, a good attempt has been made to establish the polyherbal gel consisting of Amla, Neem, Mulethi and Tulsi extracts. Initially, aqueous extracts of Amla, Neem, Mulethi and Tulsi were evaluated for various pharmacognostical parameters. The aq. The extract & of each plant was lyophilized ( $-60^{\circ}\text{C}$ ) to get powdered forms. The drug content was estimated by densitometry TLC study was carried out for testing the compatibility of drug with selected excipients used in the formulation. The approximately equivalent Rf values concluded the compatibility of Amla, Neem, Mulethi and Tulsi with the excipients. The prepared gel was characterized for various parameters i.e; Physical

appearance, viscosity, spreadability, in-vitro and ex-vivo studies. Percentage drug release study, thus conducted, revealed that about 81.110% Gallic acid (Amla), 82.006% Azadirachtine (Neem), 83.556% Glycyrrhizic acid (Mulethi) and 81.744 % Ursolic acid (Tulsi) were released from the prepared polyherbal gel. Ex-vivo drug release revealed that about 83.301% Gallic acid (Amla), 86.594% Azadirachtine (Neem), 86.989% Glycyrrhizic acid (Mulethi) and 83.413% Ursolic acid (Tulsi) were released from the prepared polyherbal gel. Finally, it was concluded that such novel preparations would be beneficial in the treatment of Mouth ulcer and applicable to other ailments pertaining to the presence of such herbal drug of different characteristics.

## 6. Acknowledgement

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