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# Formulation and Evaluation of anti-osteoarthritic and anti-inflammatory activity of Nyctanthes Arbor Tristis Linn as Emulgel

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**ABSTRACT:**
This study was performed to evaluate the various parameters to formulate the Emulgel using Nyctanthes arbor tristis leaves.The formulation contained the gelling various gelling agents and drug and evaluated for phytochemical, viscosity, pH, drug content, and in vitro drug release. The formulation was formed using Carbapol 934,Carbapol 940 and HPMC as the gelling agents. The gel and emulsion was formulated separately and mixed together at the ratio of 1:1. The results of evaluation parameters concluded that the Carbapol 934 was showed the better drug release and drug content. Although, each formulation showed the better spreadability, pH and viscosity but drug content and release was highest in the formulation (Carbapol 934).

**Keywords:** Nyctanthes arbor tristis, Anti-inflammatory, Emulgel

## I. INTRODUCTION

Nyctanthes arbor-tristis Linn, which is part of the family of Oleaceae, is a mythological plant with an extremely medicinal significance in Ayurveda. It is also known as Harsinghar in Hindi as well as parijat Sanskrit as well as night jasmine English. The ancient medicine was known for its efficacy and effect on certain diseases that cannot be ignored[1]. Similarly, Nyctanthes arbor tristis is one of them, a night blossom which contains several active phytochemical classes' chemicals which could be the main reason to pronounce its value in the modern medicine age[2]. The main effect which was researched is anti-inflammatory, which makes it the most valuable and anticipated plant for the anti-arthritis disease. A deciduous shrub or small tree from the family Oleaceae which found in the forest and vitally in home gardens generally considered as the holy tree. The seeds, flowers and leaves are more prominently used as the decoction to treat several diseases[3]. The geographical presence of this plant is more prominent from the southern Pakistan to Northern Nepal. Leaves of Nyctanthes arbor-tristis Linn, They are widely used in Ayurvedic medicines for the treatment of various ailments such as sciaticaand chronic fever, arthritis as well as internal worm infection and also as a laxative diaphoretic and diuretic.The study of phytochemistry on the leaves demonstrated that there were flavanol glycosides (astragaline and Nicotiflorin), Triterpenoid (Nyctanthic acid and Oleanolic acid) and iridoid glycosides (arborside A,B, and C) and Iridoid-glucoside (arborside D). The various components of this plant are with different therapeutic properties; however the current research is focused on the establishment of quality standards for leaf extracts of N. arbor-tristis[4].

Nyctanthes arbor-tritis Linn is a small divine decorative tree. It is domestic plant in plant; it is spread out in the wild sub Himalayan regions and southwards to Godavari. It is commonly known as night jasmine[5]. This is a deciduous tree and the growth up to 10m tall and had quadrangular branches with gray or greenish-white rough bark. In loamy soil, it grows well. The leaves are rough, hairy, opposite and simple. The arrangements of flowers are at the tip of the tree branch. Various parts of Nyctanthes arbor-tritis Linn helps for different disorders by different tribes of India such as Orissa and Bihar and also used in different medical systems like Ayurveda, Sidha and Unani[6]. The leaves of this plant are used in different medical properties such as analgesic, antipyretic, ulcer genic, Anti-stress, anxiolytic, and tranquilizing, antihistaminic and purgative property[7].

Different parts of N. arbortrisitis constitutes of various chemical compound for example Terpenes, steroids, glycosides (iridoid

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glycosides and phenylpropanoid glycosides, flavonoids, alkaloids and aliphatic. This plant produces large amount of secondary metabolites like glycosides and alkaloids[8].

## II. MATERIALS AND METHODS

### Collection of Leaves

The leaves of the Nyctanthes arbor-tristis were collected nearby locality of the ACME research solutions laboratory, Delhi. The leaves were further authenticated by the Prakash Institute. The leaves were washed thoroughly to remove the dust particles and debris of the soils. Then, the leaves were shaded dried under the closed container for minimum 15 days. The dried leaves were grinded to the powder and collected in the air tight container. The powder of the leaves was further subjected to the phytochemical screening.

### Phytochemical Screening

The phytochemical screening was done to evaluate the phytochemicals presents in the medicinal plants. The screening was done with all the three extract (Methanol, ethanol and water). The present phytochemical are likely to claim the medicinal activity. The tests were performed to see the activity of the medicinal extract of the plant (Table-1).

**Phytochemical Analysis**

| Sn. | Test | Solvents | | |
|---|---|---|---|---|
| | | Methanol | Ethanol | Water |
| 1 | Alkaloids | + | + | +++ |
| 2 | Carbohydrates | + | - | ++ |
| 3 | Saponins | + | + | ++ |
| 4 | Glycosides | + | ++ | ++ |
| 5 | Steroids | + | + | - |
| 6 | Phenol | + | + | ++ |
| 7 | Flavonoids | + | +++ | ++ |

Table-1

### Formulation of Emulge

This herbal formulation was prepared using different polymers such as Carbapol 934, Carbapol 940 and HPMC. 1% of the amount of the polymer were used and dissolved in the hot water (80ºC) using mechanical shaker and left overnight. The drug was thoroughly mixed in the water.

The Oil phase of the Emulgel was prepared using Tween 80. The Tween 80 was thoroughly mixed in the liquid paraffin wax. The oil phase was stored in separate container. The aqueous phase prepared using Tween 80 in the water. Along with that methyland propyl paraben were dissolving in Propylene glycol. The drug solution and the preservative solutions were added to the aqueous phase. Both the phases were heated and mixed together. The solution of the Emulsion and Gel mixed together at the ratio of 1:1.

The formulation was maintained the pH under the 7. The pH fluctuation was optimized by using the tri-ethanolamine. The optimized formulation was then mixed with the extract (1%). The formula followed as mentioned in the Table-2. The gel and formulation was thoroughly mixed using the magnetic stirrer at the constant temperature.

**Formulae for Emulgel**

| Sn. | Ingredients | Formulations | | | |
|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 |
| 1 | Extract | 1 | 1 | 1 | 1 |
| 2 | Carbopol 940 | 1 | NA | NA | 2 |
| 3 | Carbopol 934 | NA | 1 | NA | NA |

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| | | | | | |
|---|---|---|---|---|---|
| 4 | HPMC | NA | NA | 1 | NA |
| 5 | Liquid paraffin | 7.5 | 7.5 | 7.5 | 7.5 |
| 6 | Propylene glycol | 5 | 5 | 5 | 5 |
| 7 | Methyl Parabene | 0.03 | 0.03 | 0.03 | 0.03 |
| 8 | Propyl Parabene | 0.03 | 0.03 | 0.03 | 0.03 |
| 9 | Water | q.s. | q.s. | q.s. | q.s. |

Table-2.

**Evaluation Parameters**

**Physical Examinations**

The Physical examination were performed by sighted the formulation such as their texture, color, clarity and existence of particle (Table-3).

**Physical Examinations**

| Sn. | Formulations | Gel | Transparency | Color | Uniformity | Particles |
|---|---|---|---|---|---|---|
| 1 | F1 | 1 | Clear | Brown | Uniform | None |
| 2 | | 2 | Clear | Brown | Uniform | None |
| 3 | | 3 | Clear | Brown | Uniform | None |
| 4 | F2 | 4 | Clear | Brown | Uniform | None |
| 5 | | 5 | Clear | Brown | Uniform | None |
| 6 | | 6 | Clear | Brown | Uniform | None |
| 7 | F3 | 7 | Clear | Brown | Uniform | None |
| 8 | | 8 | Clear | Brown | Uniform | None |
| 9 | | 9 | Clear | Brown | Uniform | None |
| 10 | F4 | 10 | Clear | Dark Brown | Uniform | None |
| 11 | | 11 | Clear | Dark Brown | Uniform | None |
| 12 | | 12 | Clear | Dark Brown | Uniform | None |

Table-3

**Determination of pH**

The pH was determined by the digital pH meter. 1 gram of the formulation was taken and dissolved in the 100mL of purified water. The solution was left for 2-3 hours to insure that the formulation is settled down. The pH measurement was performed 3 times for a formulation and then took the average with standard deviation (Table-4).

**pH Determination**

| Sn. | Formulations | Triplicates | pH |
|---|---|---|---|
| 1 | F1 | 1 | 6.17±0.12 |
| 2 | | 2 | |
| 3 | | 3 | |
| 4 | F2 | 4 | 6.17±0.25 |
| 5 | | 5 | |
| 6 | | 6 | |
| 7 | F3 | 7 | 6.27±0.21 |
| 8 | | 8 | |

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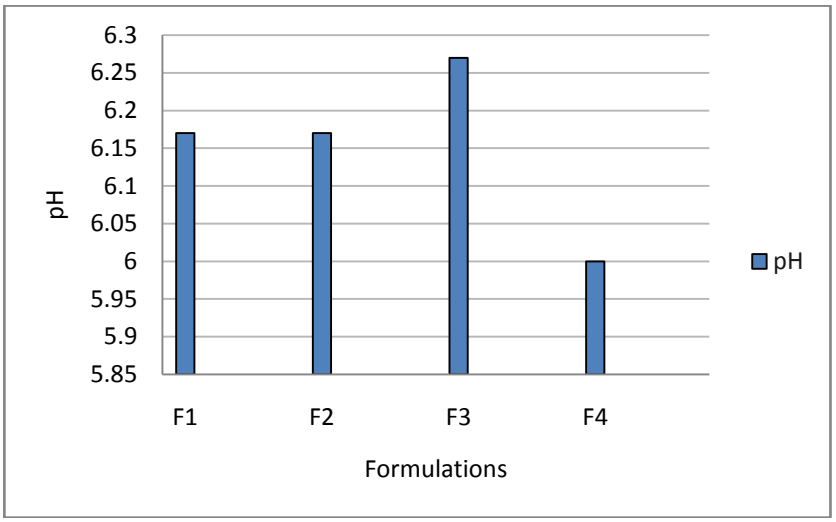
| | | | |
|---|---|---|---|
| 9 | | 9 | |
| 10 | F4 | 10 | 6.00±0.10 |
| 11 | | 11 | |
| 12 | | 12 | |

Table-4




Graph.1- pH Determination

**Viscosity**

The viscosity was measured using the viscometer (iGenelab) at the different RPM (Spindle no: 42) type) at the room temperature. The RPM were set i.e. 20, 40, 60 and 100. Each time the viscosity recorded and took the average of the readings (Table-5).

**Viscosity**

| Sn. | Formulations | Triplicates | Viscosity (cps) | |
|---|---|---|---|---|
| | | | 0.5 RPM | 1 RPM |
| 1 | F1 | 1 | 582.87±5.44 | 309.33±2.30 |
| 2 | | 2 | | |
| 3 | | 3 | | |
| 4 | F2 | 4 | 431.8±40.32 | 226.47±4.43 |
| 5 | | 5 | | |
| 6 | | 6 | | |
| 7 | F3 | 7 | 607.53±4.82 | 304.67±3.41 |
| 8 | | 8 | | |
| 9 | | 9 | | |
| 10 | F4 | 10 | 641.67±31.72 | 407.2±5.02 |
| 11 | | 11 | | |
| 12 | | 12 | | |

Table-5

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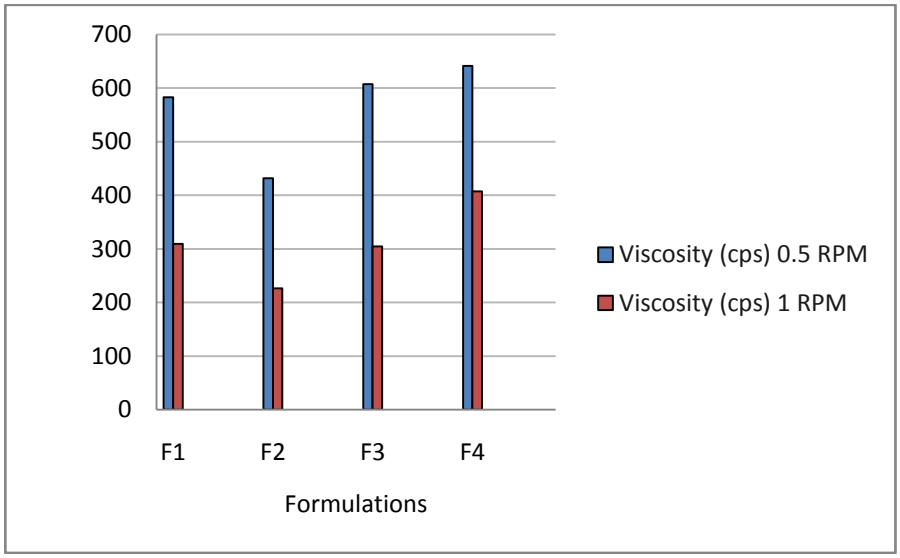


Graph.2- Viscosity for different formulations

**Spreadability**

The spreadability of the Emulgel was evaluated by using glass plates and applied the constant force. The approximate 500mg of the Emulgel was weighed and put on the plat. The other plat was forcedly dropped on to another. The circle of the spread was noted down and averages of three drops were counted (Table-6).

**Spreadability**

| Sn. | Formulations | Triplicates | Spreadability(cm/sec) |
|---|---|---|---|
| 1 | F1 | 1 | 10.59±0.56 |
| 2 | | 2 | |
| 3 | | 3 | |
| 4 | F2 | 4 | 12.49±0.45 |
| 5 | | 5 | |
| 6 | | 6 | |
| 7 | F3 | 7 | 9.87±0.68 |
| 8 | | 8 | |
| 9 | | 9 | |
| 10 | F4 | 10 | 8.92±0.68 |
| 11 | | 11 | |
| 12 | | 12 | |

Table-6

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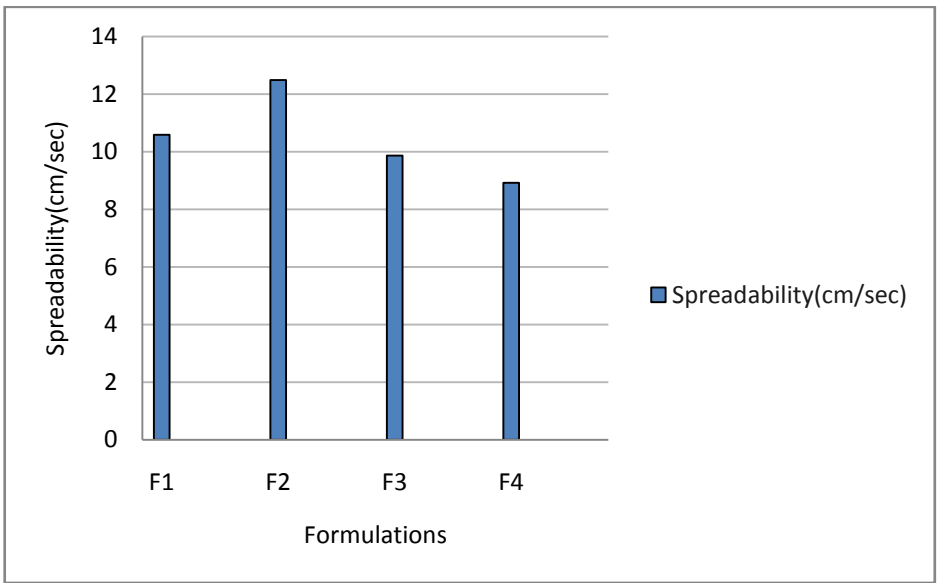


Graph.3- Spreadability for different formulations

**In vitro drug Release**

In vitro drug release studies were performed using the Franz diffusion cell apparatus. The Emulgel were taken on the dialysis membrane which was soaked in the buffer solutions. The inlet was fixed with magnetic stirrer, which was moving in a direction and making the solution warm. The compartments of the diffusion cell apparatus was filled with the 10 mL of phosphate buffered saline maintained at 6.8 pH. This process was carried out for 9 hours. Every 30 min the solution was taken and the analyzed by UV-VIS Spectrophotometer at 215nm. The samples were filled again after using the previous sample for the analysis. The cumulative drug release was evaluated using the UV-VISSpectrophotometer(Table-7).

**In vitro Drug Release**

| Sn. | Time | F1 | F2 | F3 | F4 |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | 11.01 | 10.25 | 12.24 | 11.21 |
| 3 | 2 | 28.21 | 30.11 | 27.25 | 26.54 |
| 4 | 3 | 35.02 | 39.25 | 30.28 | 32.65 |
| 5 | 4 | 51.27 | 59.82 | 50.13 | 49.39 |
| 6 | 5 | 59.88 | 61.34 | 60.89 | 58.65 |
| 7 | 6 | 62.41 | 72.98 | 68.32 | 65.02 |
| 8 | 7 | 78.96 | 85.25 | 78.32 | 70.06 |
| 9 | 8 | 90.00 | 95.66 | 89.69 | 88.35 |

Table-7

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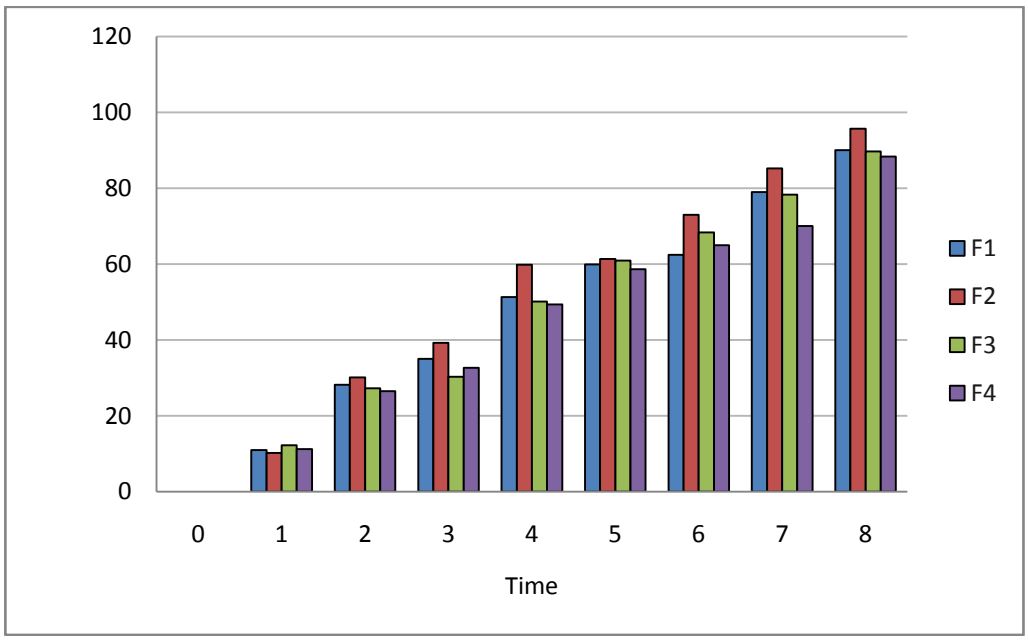


Graph.4- In-vitro drug release studies

**Drug Content**

Drug content was determined by using the 1g of the sample taken in the volumetric flask filled with the water. And the each 0.1g sample again diluted with the 10 mL of the water and filtered using filter paper. The diluted content of the Emulgel analyzed using spectrophotometrically at 215nm(Table-8).

**Drug Content**

| Sn. | Formulations | Triplicates | Drug Content at 248nm |
|---|---|---|---|
| 1 | F1 | 1 | 95.23±0.06 |
| 2 | | 2 | |
| 3 | | 3 | |
| 4 | F2 | 4 | 95.77±0.15 |
| 5 | | 5 | |
| 6 | | 6 | |
| 7 | F3 | 7 | 94.63±0.38 |
| 8 | | 8 | |
| 9 | | 9 | |
| 10 | F4 | 10 | 94.20±0.10 |
| 11 | | 11 | |
| 12 | | 12 | |

Table-8

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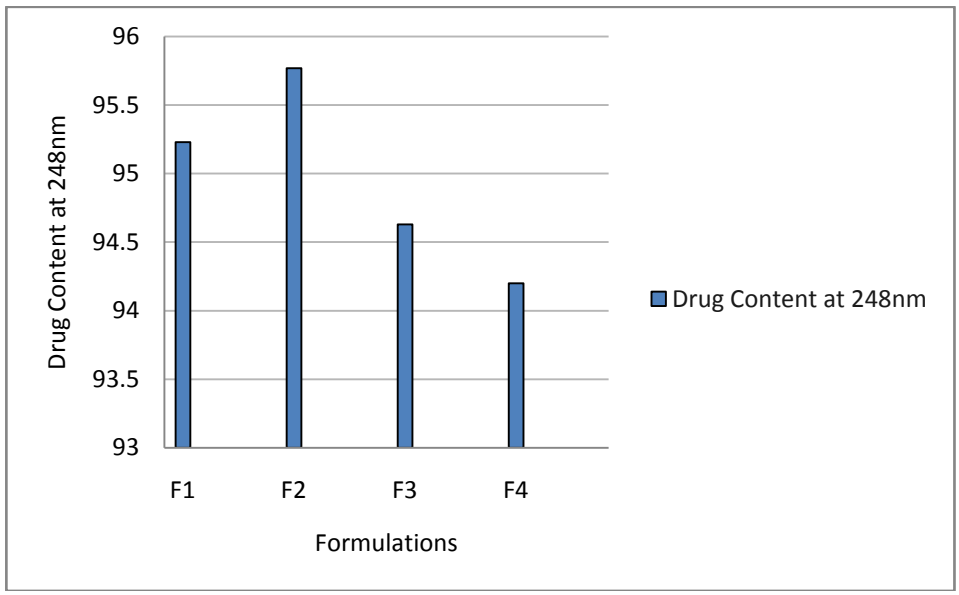


Graph.5- Drug content of different formulation

**Extrudability**

The Extrudability of the Emulgel was evaluated by the method being used for the marketed formulations of the Emulgel. The percentage of the amount displaced by the amount forced out from the aluminum tubes is considered as the extrudability of the Emulgel. The aluminum collapsible tubes were used to perform this test. This required at least 0.5cm ribbon of the Emulgel in 10s. If the more amount comes out that would considered as the excellent and if less comes out would be considered as average. This evaluation parameter was performed triplicates so that average data would be compared with the standard formulation (Table-9).

**Extrudability=Applied weight to extrude gel from tube (g)/ area (cm2)**

**Extrudability**

| Sn. | Formulations | Triplicates | Extrudability |
|---|---|---|---|
| 1 | F1 | 1 | Excellent |
| 2 | | 2 | Excellent |
| 3 | | 3 | Excellent |
| 4 | F2 | 4 | Excellent |
| 5 | | 5 | Excellent |
| 6 | | 6 | Excellent |
| 7 | F3 | 7 | Good |
| 8 | | 8 | Good |
| 9 | | 9 | Average |
| 10 | F4 | 10 | Good |
| 11 | | 11 | Good |
| 12 | | 12 | Good |

Table-9

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**Stability Studies**

Stability studies were evaluated for its stability for a definite time interval according to the International Conference on Harmonization (ICH) guidelines. Short term studies were carried out for the 3 month for all the formulations. The Emulgel samples were stored at the three different temperatures refrigerator (4-8ºC), room temperature (25±2ºC) and high temperature (45 ±2ºC). Sample were taken out from the stability chamber in every 12th week and analyzed for the physical appearance. The samples conditions were noted down which is mentioned in the table-10. The criteria of the stability studies were set upon the few parameters such as color, physical appearance and pH.

**Stability Study**

| Sn. | Formulations | Month | Appearance | pH | Drug Content |
|---|---|---|---|---|---|
| 1 | F1 | 3 | Brownish | 6.1±0.06 | 90.0±1.0 |
| 2 | F2 | 3 | Brownish | 6.3±0.06 | 91.7±0.6 |
| 3 | F3 | 3 | Brownish | 6.0±0.01 | 90.3±0.6 |
| 4 | F4 | 3 | Brownish | 6.0±0.21 | 90.0±1.0 |

Table-10

## III. RESULTS AND DISCUSSION

The leaves of Nyctanthes arbor-tristis were collected and formulated as an Emulgel to evaluate the activities i.e. arthritis and inflammation. The Emulgel was formulated with the help of the three different gelling agents and subsequently evaluated. The formulation was brown in color and viscous with smooth texture and shiny appearance. The evaluation parameters were included organoleptic properties, homogeneity, pH determination, spreadability, viscosity, extrudability, in vitro drug release and stability studies.

The phytochemical screening was done to confirm the phytochemical presence in leaves extract. The extract were identified in the all three solvents i.e. methanol, ethanol, and water. The results showed the presence of the alkaloids, carbohydrates, saponins, glycosides, steroids, phenols and flavonoids (Table-1).

The evaluation parameters were done for the gel and Emulgel. The consistency, homogeneity was excellent and good for all the formulations (Table -2). The pH of the Emulgel was measured and result showed that pH of the formulation was in range (6.00 to 6.17) (Table-4).

The viscosity for Emulgel was evaluated using the viscometer at 2 different RPM range (0.5 and 1). The results showed that the all the 4 formulation contains the constant consistency in terms of viscosity and falls between the range of maximum 641.67 at 0.5 RPM and 407.2 at 1 RPM (Table-5).

The spreadability of Emulgel was measured using the glass plate and results were satisfactory showed the uniform spread all over the glass when applied the constant force. The formulation F2 showed the better spreadability as compared to other formulations (12.49) (Table-6).

The in vitro drug release from the Emulgel was evaluated by using Franz Diffusion cell apparatus. The 6.8 pH buffer was used to assess the drug release. The process was carried out for the maximum 8h (0, 30, 1, 2, 4, 6 and 8h); every time point the drug aliquot was taken and replaced with the same amount of buffer. The sample was analyzed using UV-VIS spectrophotometer and 215nm. However, the maximum release of the drug was noted in the formulation number 2 (95.66) [F2] (Table-7).

The drug content evaluation showed the maximum in the formulation F2 (95.77) amongst all other formulates Emulgel (Table-8).

The stability studies also suggested that the F2 formulation was well stable and physical appearance was not disturbed at the various temperatures for a time period of 3month. Spreadability, pH, and drug content parameters were stable during the stability studies. The integrity and stability of the formulation F2 was remarkable and it can be concluded that the formulations were passed during all the other evaluation parameters. The results showed that F2 formulation was consistently functioned well and the best among other formulation (Table-10).

The future research may include the in vivo testing of the formulation in osteoarthritis model.

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## IV. CONCLUSION

The Nyctanthes arbor-tristis leaves herbal Emulgel was formulated using different gelling agents. Different evaluation parameter was performed to see the activity of the Emulgel. These formulations were tested for the spreadability, extrudability, viscosity, drug release and drug content. The results showed the F2 formulation prepared by using Carbapol 940 as the gelling agent showed the maximum spreadability, drug content and drug release. Hence, the Nyctanthes arbor-tristis leaves Emulgel can be prepared and beneficial as anti-inflammatory and anti-arthritis.

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