**Formulation and characterization of *Chrozophora tinctoria* phytosomal loaded gel for diabetic wound healing**

**Monika Semwal (Research Scholar, CT University, Ludhiana), Dr. Neeraj Mishra, Dr. Vir Vikram**

**ABSTRACT:** *Chrozophora tinctoria* (L.) A. Juss is an herb belongs to the family Euphorbiaceae having various medicinal and therapeutic importance. The current article covers an emphasis on the key aspects of plant Chrozophora tinctoria with regards to their phytosomal gel uses in wound healing potential.

Keywords: Chrozophora tinctoria, Euphorbiaceae, Wound, Phtosomal

**INTRODUCTION**

***Chrozophora tinctoria***

This plant primarily grows in the Mediterranean region, as well as in central and southern Asia. (1). It is native to temperate and tropical areas of Asia (such as Kuwait, Saudi Arabia, Afghanistan, Iran, Iraq, Israel, Jordan, Lebanon, Syria, Turkey, Kazakhstan, Turkmenistan, India, and Pakistan), Africa (including Algeria, Egypt, Libya, Morocco, Tunisia, and Yemen), and parts of Europe (1).

Chemical Components of Chrozophora tinctoria:A preliminary examination of Chrozophora tinctoria (whole plant%) revealed that it contained 50.00, organic material 92.73, protein from crude sources 9.13, neutral fiber for detergent 31.06, and acid detergents fiber 54.10 (2). An investigation revealed that Chrozophora tinctoria contains dyes, alkaloid compounds, a group of compounds coumarins, chromes, diterpenoids, and phenylpropanoid glycosides. Chrozophora tinctoria stems, leaves, and seeds were gathered in winter from two habitats in Sinai, Egypt, and analyzed to reveal the presence of the tannins, flavonoids, phenolics, alkaloids, glycosides, reducing sugars, chlorides, and sulfates. The presence of free arabinose, ribose, fructose, glucose, and raffinose, as well as mixed sucrose, was determined using HPLC. Additionally, eight amino acids were removed from the design (2).

**Classification of Chrozophora tinctoria**

* Kingdom: Plantae,
* Subkingdom: Tracheobionta,
* Superdivision: Spermatophyta,
* Division: Magnoliophyta,
* Class: Magnoliopsida,
* Subclass: Rosidae,
* Order: Euphorbiales,
* Family: Euphorbiaceae,
* Genus and Botanical Authority: Chrozophora and (Linnaeus) A. Jussieu
* Species: Chrozophora tinctoria (3).

**Diabetes**

Diabetes mellitus is a condition which distresses the person’s ability to control their own blood sugar level because their body doesn’t produce enough insulin or because of insulin resistance when cells don’t respond to the insulin that is produced. High blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst), weight loss and lethargy (4).

**Phytosomes**

Phytosomes are very recent introduction into herbal formulation as they are better absorbed and have higher bioavailability. The term phyto means “plant” while “some” means cell like. This are advanced forms of herbal formulation that contains bioactive phytoconstituents of herbal extract surrounded by a lipid. Phytosomes have a better pharmacokinetic and pharmacodynamic profile (5)

 Advantages of phytosomes are increased bioavailability, efficient nutrient safety, and optimum entrapment efficiency (6)

**Wound**

Wound healing is a complicated progression wherever the skin or further body tissue maintains itself behind injury. The epidermis and dermis layer of the skin appearance a defensive obstruction at the side of the exterior atmosphere (7). This procedure is divided passionate in the direction of expected phases: blood clotting (hemostasis), inflammation, the enlargement of new tissue (proliferation), and the remodeling of tissue (maturation). From time to time blood clotting is measured to be fraction of the inflammation step instead of its own phase (8).

**METHODS OF PREPARATION OF PHYTOSOMES**

**Preparation of plant extract**

The plant leaves were left to air dry at room temperature and out of direct sunlight for 24 hours. After drying, they were ground into a fine powder with a laboratory grinder. Subsequently, the powdered leaves were extracted with ethanol using a Soxhlet extractor. The ethanol was then removed and purified using a rotary evaporator and water bath. The resulting dried extracts were kept in the refrigerator for future analysis.

**Phytosome preparation**

*Chrozophora tinctoria* plant extract was used to make test batches of phytosome complexes using a number of processes, such as antisolvent precipitation, rotary evaporation, and solvent evaporation.

**FORMULATION OF PHYTOSOMES OF *CHROZOPHORA TINCTORIA***

Thin film hydration was used to create phytosomes utilizing varied medication molar ratios, 30% *Chrozophora tinctoria*, soya lecithin and cholesterol. The medication was dissolved in methanol, while precisely weighed amounts of *Chrozophora tinctoria* and cholesterol were dissolved in dichloromethane. The aforementioned solution was placed in a round bottom flask, vaporized in a rotary evaporator at 40°C at 180 rpm, and vacuumed until all of the solvent had evaporated (8).

A thin layer was then obtained and added to the RBF. The flask spent up to 24 hours in the refrigerator. The film was hydrated in a rotary evaporator at 40°C for 1 hour using a 1:1 combination of ethanol and water. After making the phytosomal suspension, the mixture was sonicated for 30 minutes to minimize the particle size. A phytosomal preparation made from *Chrozophora tinctoria* and made with cholesterol exists.

**Calculation of max**

A stock solution with a concentration of 10 mg/ml was prepared by dissolving about 10 mg of pure *Chrozophora tinctoria* extract in 10 ml of phosphate buffer at pH 7.4. This stock solution was then diluted by adding 10 ml to 100 ml of the same buffer. The resulting solution was analyzed within the 200–400 nm wavelength range to determine the optimal absorbance. Further analysis was performed at the selected wavelength of 330 nm.

**Assessment of Phytosome**

A close-up view optical microscopy was used to identify the phytosome. While the phytosome was suspended in buffer, a drop of it was placed on a slide and covered with a cover slip. The phytosome was visible in a microscopic form with a 45X magnification.

**Drug identification**

*Chrozophora tinctoria* absorbance spectra in 10% ethanolic potassium hydroxide a 100mg/ml stock solution of *Chrozophora tinctoria* was produced in 10% ethanolic potassium hydroxide, and it was scanned using a UV double beam spectrophotometer (Shimadzu, Japan) in the 400–800nm wavelength range.

**Gel preparation:**

By independently dispersing Carbopol 934 in distilled water with continual stirring at a reasonable speed while using a mechanical shaker, gel bases were created. Using triethanolamine, the pH of all the formulation was brought down to 5.5 to 6.5.

**CHARACTERIZATION OF PHYTOSOMAL GEL**

**Formulation of Chrozophora Tinctoria**



**Calibration Curve of *Chrozophora tinctoria* Extract**

By scanning the produced solution in the 200–400 nm wavelength range, the maximum amount of *Chrozophora tinctoria* Extract was identified. It was discovered that the maximum wavelength was 330nm. Dissolving the medication in phosphate buffer at pH 7.4 allowed for the construction of the calibration curve for Chrozophora tinctoriaextract. The concentration range of 2–10 mg/ml was where the curve was determined to be linear. The regression coefficient (R2) value was found to be 0.9942

**Percentage Practical Yield of formulations**

The % Practical Yield of various formulations. MF3 has a greater % Practical yield of 90.74% compared to other preparations.

|  |  |
| --- | --- |
| **Formulation** | **Percentage Yield of formulations** |
| **MF1** | **86.45** |
| **MF2** | **88.43** |
| **MF3** | **90.74** |
| **MF4** | **89.21** |
| **MF5** | **88.37** |
| **MF6** | **89.70** |
| **MF7** | **86.45** |

**Entrapment Efficiency**

**% Yield of Entrapment Efficiency (EE)**

**Percent** **Yield of Entrapment efficiency**

|  |  |
| --- | --- |
| **Formulations** | **% yield of EE** |
| **MF1** | **85.40** |
| **MF2** | **87.42** |
| **MF3** | **89.66** |
| **MF4** | **88.26** |
| **MF5** | **82.41** |
| **MF6** | **79.43** |
| **MF7** | **71.74** |

**Drug Content**

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| --- | --- |
| **Formulation** | **Drug Content (%W/W)** |
| **MF1** | **89.23** |
| **MF2** | **90.42** |
| **MF3** | **91.02** |
| **MF4** | **88.66** |
| **MF5** | **87.34** |
| **MF6** | **83.10** |
| **MF7** | **79.60** |

In comparison to the pure substance extract, *Chrozophora tinctoria* phytosomes considerably displayed an improved diffusing behavior. Most of the preparations demonstrated an average amount of drug released in the range of 79.30 - 92.45%, in contrast to the absence of drug extract, which showed a total of just 61.23% drug release after 10 hours. At the 10th hour, the formulation MF3 with a drug extract: soy lecithin ratio of 1:1 demonstrated the greatest release of 92.45%. The complex process of pharmaceutical molecules diffusing from their form of administration depends on a wide range of variables, including particle size, crystallographic routine surface area, surface energy, and moisture absorption. The soluble portion of the medicine was boosted because to the absorbing and dispersing capabilities of phospholipids (an amphiphilic surfactant), which also improved its absorption characteristic.

**Viscosity of Formulations**

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| --- | --- |
| **Formulations** | **Viscosity (Cp)** |
| **MF1** | **397±20.62** |
| **MF2** | **410±23.30** |
| **MF3** | **451±.22.14** |
| **MF4** | **497±12.41** |
| **MF5** | **510±35.72** |
| **MF6** | **600±26.84** |

**Spreadability of phytosomal gel**

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| --- | --- |
| **Formulations** | **Spreadability (cm)** |
| **MF1** | **2.9** |
| **MF2** | **3.2** |
| **MF3** | **4.7** |
| **MF4** | **3.8** |
| **MF5** | **3.1** |
| **MF6** | **4.2** |

###  Solubility Determination

The phytosomes from Chrozophora tinctoria were shown to be considerably more soluble than the pure plant extract. The solubilization theory resulting from the production of micelles in the medium of choice and the complex's amorphous character can both be used to explain the increase in solubility of the plant extract in the system. By virtue of their wetting and dispersion qualities, these amphiphilic surfactants (phospholipids) may improve the solubility of the plant extract. The most solubility was seen in the formulation MF3.

 **Solubility in different solvents**

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| --- | --- | --- | --- |
| Formulation | Water(mg/ml) | PhosphateBuffer(mg/ml) pH7.4 | n-Octanol(mg/ml) |
| Plant Extract | 0.452 | 0.547 | 0.591 |
| MF1 | 0.561 | 0.573 | 0.642 |
| MF2 | 0.584 | 0.577 | 0.620 |
| MF3 | 0.601 | 0.624 | 0.648 |
| MF4 | 0.552 | 0.580 | 0.599 |
| MF5 | 0.512 | 0.534 | 0.586 |
| MF6 | 0.536 | 0.537 | 0.570 |
| MF7 | 0.504 | 0.510 | 0.549 |

**CONCLUSION**

By analyzing the solution produced within the 200–400 nm wavelength range, the researchers successfully pinpointed the presence of a significant amount of Chrozophora tinctoria Extract. Through meticulous examination, it was specifically noted that the peak absorption occurred at a wavelength of 330nm. The team then proceeded to dissolve this medicinally valuable extract in a phosphate buffer with a pH level of 7.4 to facilitate the creation of a precise calibration curve specifically tailored for Chrozophora tinctoria extract. During the calibration process, the researchers determined that the concentration range falling between 2 and 10 g/ml was ideal, as this interval displayed a linear relationship between concentration and absorbance values. The correlation between the two variables was further established with a high level of confidence given the regression coefficient (R2) value of 0.9942 obtained from the conducted experiments.

The range provided, from 91.02% to 79.60%, pertains to the drug content found within the Chrozophora tinctoria extract formulations, showcasing a satisfactory drug level

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