



Design, Formulation, and Physicochemical Evaluation of *Occimum sanctum* Containing Honey Based Hydrogel

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i34B31846

Editor(s):

(1) Dr. R. Deveswaran, M.S. Ramaiah University of Applied Sciences, India.

Reviewers:

(1) Fatma Zaghoul Mahmoud Abdl Razik, Cairo University, Egypt.

(2) Zenati Latifa, Université Benyoucef Benkhedda d'Alger 1, Algeria.

complete Peer review History: <http://www.sdiarticle4.com/review-history/69944>

Original Research Article

Received 25 April 2021

Accepted 29 June 2021

Published 01 July 2021

ABSTRACT

Background and Aim: The present study aims to formulate a hydrogel containing Tulsi (*O. sanctum*) and honey, to obtain a pharmaceutical topical preparation having desirable healing effects and antibacterial properties of both the ingredients.

Material and Method: Topical honey hydrogel formulation were prepared using 75%honey and 10% *Occimum sanctum* (Tulsi) with polymer carbopol 934. The prepared formulation was assessed for its pH, spreadability, swelling index and *in-vitro* release

Result: The pH and spreadability were in the range of 4.74 ± 0.02 and 7.75 ± 0.04 cm respectively. Carbopol based Honey –Tulsi Formulation showed a concentration-dependent increase in the amount of honey and *O. sanctum* diffused through dialysis method.

Conclusion: Within the limitations of the study, the results of our present study suggest that the studied Honey –Tulsi based hydrogel could be an economic indigenous substitute which is non-toxic, natural and efficient for clinical application.

Keywords: Honey; *Ocimum sanctum*; hydrogel.

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1. INTRODUCTION

Honey is defined as concentrated solution of sugar prepared by bees from the nectar of plants. Humans have consumed honey for thousands of years due to its unending health benefits. Honey is a sweet syrup viscous in nature and contains carbohydrates, proteins, amino acids, vitamins and minerals. In ancient literature it has been well documented for therapeutic purposes owing to its evidence based antimicrobial [1-2], and analgesic [3] property .It is also used to combat respiratory tract infection [4].

In Ayurveda, Tulsi .i.e. holy basil is considered as “The Queen of Herbs” and also “The mother Medicine of nature”. In India, Tulsi has been adopted into daily lifestyle practices and spiritual rituals that provide variety of health benefits to our body [5]. Tulsi .i.e. Oil of *O. sanctum* possess anti inflammatory properties due to its rich content of vitamin C, beta carotene and calcium. It acts by inhibition of cyclo-oxygenase and lipoxygenase pathways, thereby modulating cyclo-oxygenase pathways and lipo-oxygenase pathway. It helps in modulating humoral and cell mediated immune response [6]. The Leaves of this herb has unique property as they are diaphoretic and antiperiodic so used in patients suffering from bronchitis, gastric and hepatic disorders [7].

2. PROPERTIES OF TULSI

- I. anti inflammatory
- II. Anti-oxidant
- III. Antidiabetic,
- IV. Immunomodulatory
- V. Cardio protective
- VI. Hypolipidemic
- VII. Antistress,
- VIII. Antifertility, and
- IX. Anticancer properties [8]

due to which it acts as a prospective agent for treating wide array of disease ranging from mild illness to severe conditions.

Honey as we all know is viscous in nature its direct application on an affected area remains complicated. The main disadvantage is leakage and liquefaction that leads to difficulty in maintaining the therapeutic concentration for a periodic time over the affected region.

To overcome the above mentioned limitations we incorporated honey in a hydrogel formula that is reported to deliver drug for sufficient time. It can be used for medical purpose, wound management in particular [9-11,12] owing to its characteristic property of humid environment and good fluid absorbance property that is essential for successful wound healing process and aids in pain management [9].

Thus the present study focuses at overcoming the disadvantages of direct application of honey by formulation of Tulsi containing honey-based hydrogel, to finally obtain a pharmaceutical honey topical preparation with tulsi for desirable healing effects and antibacterial properties.

3. MATERIAL AND METHODS

3.1 Honey Samples

Honey was purchased from local Stores of Nagpur ,Maharashtra, carbopol 934TM (Goodrich Chemicals,USA) and Triethanolamine (TEA)TM and Methyl parabenTM were purchased from (Fisher Scientific, U.K). All the chemicals used were of analytical Grade.

3.2 *O. sanctum* (Tulsi) Samples

The fresh leaves of tulsi were procured from local market of Nagpur and approved by a botanist from Department of Botany, Dharampeth Science College, Nagpur, Maharashtra.

3.3 Preparation of 10% *Occimum sanctum* Extract

The Tulsi (*O. sanctum*) leaves were cleaned under running tap water and shade dried under sunlight for 24 hours. The sun dried leaves were stocked up in an uncontaminated sealed container until further use.

Tulsi extract was prepared by using Soxhlet apparatus by addition of 100gms of dried tulsi in the thimble followed by 500ml of double distilled water as a solvent so as to obtain 10% concentration. The process was continued till the solvent appeared in the thimble, further the extract was placed in a digital water bath till a dark green residue was obtained. The extract was stored in a sterile sealed container until further use [6].

3.4 Preparation of 75% Honey Based Hydrogel containing *Ocimum sanctum*

Hydrogel was prepared using cold mechanical method by dissolving a predefined amount of carbopol polymer in purified water with constant stirring using a magnetic stirrer for an hour till its complete dispersion in the water followed by addition of TEA so as to neutralize the pH of the hydrogel.

Methyl paraben was further added as a preservative. The Mixture was then kept in room temperature for 24 h so as to attain equilibration of the polymer and notable puffiness.

Finally 75% honey was added to the aforementioned mixture with constant stirring till it is completely disseminated in the hydrogel [13].

The final weight was completed to 100 g with the aqueous solution. At this point 10% *O. sanctum* extract was dispersed separately in the aqueous solution and stirred unless a homogenous mixture was obtained.

The final formulation were stored in a glass container and covered with screw plastic lid and sited in refrigerator to complete the formation of Honey – Tulsi hydrogel .

3.5 Physicochemical Evaluation of Honey Hydrogel Formulae

3.5.1 Visual examination

The prepared Honey –Tulsi hydrogel formulation was examined by visual check for their color, consistency, homogeneity and existence of lumps after they were set in the container.

3.5.2 pH determination

One g of the hydrogel formulae was weighed and mixed with 25 ml of purified water. The pH of the formulation was measured using a precalibrated pH meter™ (Orion Research Inc. USA), with buffer solution having pH 4.00/7.00/9.00 (25 °C) .Experiments were carried out for three readings and average values were taken into consideration.

3.5.3 Swelling index

Swelling index of the prepared topical tulsi – honey hydrogel, was calculated by soaking one

gram of the hydrogel into 5 ml phosphate buffer (pH 5.5) and left in place for a detailed time, followed by removal of excess buffer .The hydrogel was weighted again. This test was done at time intervals after 1 and 3 hours. The following formula was used to calculate the swelling ratio [14].

$$\text{Swelling ratio} = (W_s - W_t/W_0) \times 100$$

where W_s is the weight of the swollen hydrogel at time t and W_0 is the initial weight.

3.5.4 Spreadability measurement

The spreadability of the hydrogel formulation was determined by the method suggested by M. H. Shukr and G. F. Metwally [15]. According to which, 0.5 g prepared hydrogel was pressed between two horizontal plates (20 × 20 cm), followed by addition of 5 g standardized weight on the upper plate and left for about 5 minutes unless no more spreading was expected. Diameters of spread circles were measured in cm and were taken as comparative values for spreadability. The results obtained were the average of three observations.

3.5.5 In vitro Drug release studies

Honey and *O. sanctum* release from prepared hydrogels was studied using a dialysis method [16].

Presoaked Dialysis bags (Spectra/PorR Dialysis Membrane, MWCO:3.500, Spectrum Laboratories Inc., USA) were inseretd in distilled water at room temperature for 12 h for removal of any preservative, followed by thorough rinsing with distilled water.

A specific weight of the hydrogel was placed in a measured volume (100 ml) of 5.5 pH phosphate buffer at 37 ± 0.5 °C and the quantity of released honey and *O. sanctum* was measured at different time intervals by taking the absorbance of test sample using UV-Vis spectrophotometer (Shimadzu 1800, Japan).

The absorbance of the released honey was measured at λ max 340 nm.

All experiments were done in triplicates, the average of results were taken. Blank experiments were done using plain bases.

4. RESULTS

4.1 Composition (%w/w)

The hydrogel composed of Honey (75%), *O. sanctum* (10%), carbopol 934 (1%), Methyl Paraben (0.1%), triethanolamine (q.s), purified water (100%).

4.2 Visual Examination

The hydrogel appears homogenous and brownish green in colour.

4.3 pH Examination

The pH of the hydrogel was found to be 4.74 ± 0.02 .

4.4 Swelling Index

Swelling ratio w/w after 1 and 3 h were $36\% \pm 0.20\%$ and $44\% \pm 1.15\%$

4.5 Spreadability

Spreadability of the formulation was found to be 7.75 ± 0.04 cm

4.6 In vitro Release

The results (Fig. 1) showed a concentration-dependent increase in the amount of honey and *O. sanctum* diffused through cellophane bag of the hydrogel formulation. It was also observed

that the release of honey and *O. sanctum* increased with increasing its concentration in the formulation.

5. DISCUSSION

The present formulation uses Tulsi at 10 % and honey at 75% concentration owing to achieve their utmost antibacterial and enhanced tissue regeneration properties as evident from studied literature [6,14].

Hydrogels have been widely used due to its sustained delivery of a variety of local therapeutic agents [13,14]. It is formulated by cross linking of polymers representing a high water content material. Carbopol, a synthetic polymer has been used in our study, owing to its characteristic controlled delivery of therapeutics and array of biological responses and mechanical strengths [17-18]

Our results proved that the process employed to prepare hydrogel formulations in this study was efficient. It was capable of producing formulations with uniform drug content and minimal variability. Both Honey and *O. sanctum* were dispersed uniformly throughout the hydrogel.

Water-soluble linear polymers like Carbopol are chemically cross-linked to formulate the hydrogel product. The process of making polymer-based physical gel is simple and easy. It involves gentle mixing of the gel components under the appropriate conditions [14].

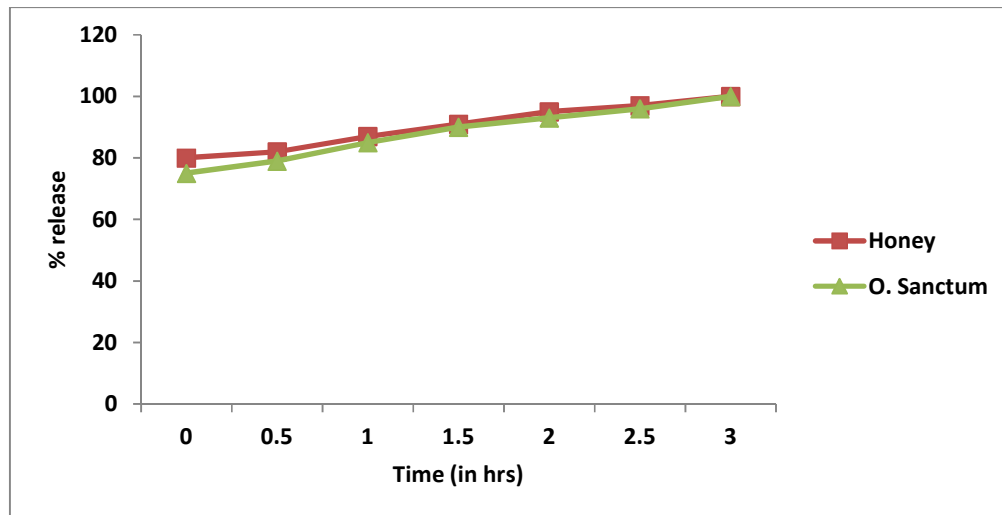


Fig. 1. concentration-dependent increase in the amount of honey and *O. sanctum*

This process has an advantage over other crosslinking methods since,

- it can be performed at room temperature,
- in physiological pH without using toxic &
- hard to remove crosslinking agents [18].

Hence the same method has been employed in our study as it is always safe for clinical applications. Hence we have observed uniform drug content and minimal variability of both Honey and *O. sanctum*.

The pH of formulated hydrogel was found to be in the range of 4.74, making it slight acidic. This could be due to the acidic property of honey itself; pH of which being between 3.2 and 4.5 [18].

Spreadability is important component in patient compliance as it helps in uniform application of gel to the surface [19]. Spreadability of our formulation was found to be 8.6 ± 0.03 making it an ideal spreadable gel for application.

Swelling index was performed for the formulation initially after 1 h and up to 3 hrs. It was noticed that our formulation showed rapid swelling due to the porous nature of the hydrogels offering large surface area allowing rapid uptake of the solvent but it was found that swelling was reduced. This finding could be attributed to the presence of the honey and carbopol as both the components are viscous in nature. Our findings correlated the relation between swelling and type of the polymer, degree of cross-linking, ionic strength and water content [20].

This is in agreement with our results as the swelling index value was found to be 36% to 44%w/w owing to the presence of carbopol.

In vitro release study showed the release of honey and *O. sanctum* from the hydrogel resulting in a concentration-dependent increase in the amount of extract diffused through cellophane bag of hydrogel formulation. The results could be attributed to the property viscosity of the gel matrix in the hydrogel.

Our Formulation showed superior and higher sustained drug release, which could be attributed to the property of polymer Carbopol 934. The component is a hydrophilic polyacrylic acid polymer and its carboxyl groups become highly ionized after neutralization with TEA, forming a

gel due to electrostatic repulsion among charged polymer [14].

6. CONCLUSION

Within the limitations of the study, the findings of our *in vitro* test suggested that the studied Honey –Tulsi based hydrogel could be an economic indigenous substitute which is non-toxic, natural and efficient for clinical application.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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