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"Formulation And Evaluation Of Ocimum Sanctum Loaded Microsponge Based Gel For Topical Delivery."

Miss. Rutuja S. Gavali[1] ,Mr. N.K.Mogal[2]

Department Of Pharmaceutics, JSPM'S Jaywanrao Sawant College Of Pharmacy & Research, Pune, Maharashtra, India.

Email ID : rutugavali98@gmail.com

ABSTRACT :

In the present work, formulate and evaluate ocimum sanctum loaded microsponge based gel for antifungal activity. Microsponge varied with drug, polymer ratios were prepared by quasi emulsion solvent diffusion method. The absorbance maxima of ocimum sanctum were found to be 284nm. FTIR study of physical mixture (i.e. drug, polymer, PVA) showed all the characteristic peaks of drug and polymer and it revealed the major functional group of drug hence there is no interaction between drug and polymer. Prepared microsponges were studied for particle size (µm), entrapment efficiency (%), production yield, scanning electron microscopy (SEM). Particle size of optimized batch (F5)was 88.462µm and SEM revealed the result that microsponges are porous and spherical in shape. Entrapment efficiency of optimized batch (F5) was found to be 69.12%. The optimized formulation of microsponges was then incorporated into gel by using carbapol934 as a polymer. Characterization of gel shows better results as pH in range 5.2-5.4, spredability was 7.2 gcm/sec and viscosity was found to be 39890 cps. Minimum inhibitory concentration(MIC) of ocimum sanctum shows antifungal activity in the concentration 20mg/ml .The comparative antifungal study was performed in ocimum sanctum plain gel and ocimum sanctum microsponge loaded gel by agar well diffusion method by using *candida albicans* species. It shows that ocimum sanctum loaded microsponge based gel having better zone of inhibition than ocimum sanctum plain gel. Hence, encapsulation of ocimum sanctum in microsponge based gel resulted in efficacious carrier system in terms of stability as well as safety of this herbal extract along with handling benefits.

Keywords: Ocimum sanctum, microsponges, *C. albicans*, gel, antifungal.

INTRODUCTION :

Candidiasis is common fungal infection caused when the skin is infected with the fungus *Candida albicans*. This form of infection is quite widespread on the skin, accounting for roughly 70% of all infections caused by this fungus. It can affect any part of the body, but it is more common in warm, wettest, wrinkled areas like the armpits and groin. Candidiasis of the skin usually manifests itself as a red, itchy rash in the skin folds. The rash could spread to other parts of your body. While the symptoms can be annoying, they can usually be managed with improved cleanliness with antifungal lotions or powders. But these

synthetic antifungal medications have negative side effects so in order to overcome herbal medicines are essentials.

Ocimum sanctum L. is herb belongs to family Lamiaceae. The leaves of plant can be used for antifungal activity. "The elixir of life" name given to *ocimum sanctum (O.Sanctum)* because it promotes longevity. It has potent antifungal, anti-inflammatory, immuno-modulatory, antioxidant, anti fertility effects. Antifungal microsponges in topical formulation considered as an effective treatment against candidiasis. In this, ethyl cellulose used as a polymer as it is hydrophobic in nature, soluble in ethyl acetate and non reactive. Polyvinyl alcohol used as emulsifying agent and has excellent film forming property. Microsponges (MS) are patented polymeric delivery systems consisting of porous microspheres that can entrap a wide range of active ingredients, it gives targeted drug delivery, sustain release of drug and increase stability of formulation.[1,2]

MATERIALS AND METHODS:

Materials: *Ocimum sanctum* was collected from local garden, Pune, Maharashtra, India. Ethyl cellulose, ethanol, ethyl acetate was supplied by S.D. fine chemicals, Mumbai; polyvinyl alcohol (PVA), triethanolamine, carbapol943 were supplied by loba chemicals, Mumbai; all other chemicals and solvents were of analytical grades.

Methods:

Authentication of Ocimum sanctum plant:

Fresh leaves of *O sanctum* were collected from the local garden. Then authentication was done at Maharashtra association for the cultivation of science, Agharkar research institute, G.G. Agharkar road, Pune-411004 under the scientist Dr. R. K. Choudhary. (AUTH 22-36).

Preparation of *Ocimum sanctum* extract:

Fresh leaves of *O. sanctum* collected and dried in shade and then the powdered the leaves and the sample was weighed on electronic balance. The powdered leaves were soaked with 200 ml of ethanol for 2 days and the residue again soaked with the same solvent (ethanol) for 24 h. The final extract was collect and filtered.[7]

Preliminary physiochemical screening:

Standard methods are employed to carry out physiochemical screening of ocimum sanctum extract. Presence of substances like carbohydrates, glycosides, flavonoids, saponins, alkaloids, phenolic compounds, tannins, terpenoids are found in the extract.[14]

UV visible spectroscopy:

The UV spectrum of *O. sanctum* extract in ethanol indicated λmax at 284 nm, the calibration curve of *O. sanctum* extract in ethanol was found linear over the range of 2 µg/ml to 10 µg/ml.

Determination of minimum inhibitory concentration (MIC) of *O. sanctum* extract:

For this, various concentration of *O. Sanctum* extract (10mg/ml to 0.01 mg/ml) were prepared in ethanol & MIC determined by broth dilution method. *O. sanctum* extracts MIC value (20mg/ml) against *Candida albicans* indicates, extract can be used for the treatment of candidiasis.[7]

Preparation of *Ocimum sanctum* loaded microsponges:

The drug-loaded microsponges were made using a quasi emulsion solvent diffusion method, solvent diffusion approach similar to that of an emulsion. Ethyl cellulose was dissolved in ethyl acetate using ultrasonication with 100 ml distilled water for 1 hour at 35°C. After that, the inner phase is put into a water-based polyvinyl alcohol solution. To evaporate ethyl acetate, the mixture was agitated at 2000 rpm for 4 hours at room temperature using a magnetic stirrer. The microsponges were collected and filtered using Whattman filter paper, rinsed in distilled water, and dried in an air heated oven.[11]

Table no.1: composition of trial batches of ocimum sanctum microsponges :

Batch code	*Ocimum sanctum* extract	Ethyl cellulose	Ethyl acetate	Triethyl citrate	Polyvinyl alcohol	Water
F1	0.2	0.1	0.1	0.01	0.25	100
F2	0.2	0.2	0.1	0.01	0.25	100
F3	0.2	0.3	0.1	0.01	0.25	100
F4	0.2	0.1	0.1	0.01	0.5	100
F5	0.2	0.2	0.1	0.01	0.5	100
F6	0.2	0.3	0.1	0.01	0.5	100
F7	0.2	0.1	0.1	0.01	0.75	100
F8	0.2	0.2	0.1	0.01	0.75	100
F9	0.2	0.3	0.1	0.01	0.75	100

Note : all values are in %

Evaluation of *Ocimum sanctum* loaded microsponges :

1. Particle Size:

Particle size of prepared microsponges was evaluated using Motic digital microscope (Image plus 2.0 software).

2. % Entrapment Efficiency (EE):

Exactly weighed (10 mg) of microsponges containing drug was kept in 100 ml phosphate buffer solution (pH 5.4) for 12 h with constant stirring. Filtered samples (using 0.45 µm disc filter. 1ml solution was With drawn and diluted up to 10 ml phosphate buffer solution (pH 5.4) and was measured UV spectrophotometrically at 274 nm against the blank solution similarly treated. Formula for % entrapment efficiency :

$$\%EE = \text{actual drug content} \div \text{amount of microsponges} \times 100$$

3. Percentage Yield (%):

The formed microsponges were washed, dried and weighed accurately. The yield of microsponges was determined by comparing the whole weight of formed microsponges against the combined weight of polymer and drug components.

$$\%\ \text{yield} = \text{mass of microsponge obtained} \div \text{total mass of drug \& polymer used} \times 100.$$

4. Scanning Electron Microscopy (SEM):

Electron micrograph of microponges powder was obtained using a scanning electron microscope (JEOL JSM-5200) operating between 5 and 24kV. The specimens were mounted on a metal stub (with double side adhesive tape) and coated under vaccum with gold in an argon atmosphere prior to observation.[1,11]

Preparation of *Ocimum sanctum* loaded microsponge based gel:

A precisely weighed quantity of Carbopol 934 was dissolved in a few ml of distilled water and placed aside in another beaker, followed by microsponges equal to the required amount of medication in the final formulation and propylene glycol. With steady stirring, the microsponge-solvent mixture was added to the inflated Carbopol above. Triethanolamine was added drop wise to the entire mixture until a translucent gel was produced. To make up the required volume, water was added. To make up the needed volume, stirring was added. Stirring was halted to allow trapped air to escape; the resulting gel was degassed with ultra sonication and stored in an airtight container for further research.[13]

Evaluation of O. sanctum loaded microsponge based gel :

1. Physical examination:

The color, texture, and appearance of the created formulations were examined visually All of the created preparations were much clearer and transparent.[4]

2. pH- determination by using pH –meter:

A digital PH-meter (type 355M/s Systronic, India) was used to determine the pH of the formulation. Before each usage, the pH meter was calibrated with a standard pH 4, 7, and 9 buffer solutions. Each formulation's pH was measured three times and the average values were calculated.

3. Homogeneity test:

100 mg of gel was pushed between the thumb and index finger to assess the consistency and texture of the gel, as well as the presence or absence of coarse particles on the finger.

4. Viscosity determination:

The viscosity of the formulated batches decided by employing Brookfield viscometer with spindle no.64 at 100 rpm.

5. Spredability:

Spread ability of gels was determined by Arvouet Grand Method by pressing 1 g of a sample between two 20 X 20 cm horizontal plates, the upper of which weighed 125 g. The spread diameter was measured after 1 min.

6.*In vitro* drug release (%):

Using a Franz diffusion cell apparatus and hens egg membrane, the release of ocimum sanctum (in vitro) from a gel containing an ocimum sanctum loaded microsponge formulation was determined. Release of *O. sanctum* (*in vitro)* from gel containing *O. Sanctum* loaded microsponges (F5) was estimated using Franz diffusion cell apparatus & pH 7.4 phosphate buffers. A sample was withdrawn at different time intervals of 0, 1, 2, 3, 4, 5 hrs. & analyzed simultaneously by UV spectrophotometry at 284 nm.

7. In vitro antifungal activity study: [15]

The fungi culture *Candida albicans* (MTCC No. 854) was used as the test strain, which was procured from the MTCC. These inoculums were spread onto nutrient agar plate by streak plate method. Reconstituted gel was tested for antifungal activity using agar diffusion on solid media.

RESULT AND DISCUSSION:

1. Preliminary physiochemical screening :

In the preliminary physiochemical testing of O. sanctum ethanolic extract substances like alkaloids, carbohydrate, tannins, flavonoids and terpenoids are present.

2. FTIR studies :

In this, 1) IR spectra of ethyl cellulose C-O-C =1118.51 cm−1, C-C=3083.62 cm−1, O-C2H5=2925.48 cm−1 functional groups are present.

2) The FT-IR spectroscopy was carried out to study the possibility of chemical interaction between ocimum sanctum with Ethyl cellulose and polyvinyl alcohol (PVA). The FT-IR spectra of isolated O. sanctum showed a characteristic N-H Stretch band 3356.25cm−1, -C-H- Aldehyde at3356.25cm−1 and –OH 3541.42cm−1 Among the various bands, the spectrum of Ethyl cellulose at 1057.03cm−1, 1643.41cm−1 for C-C-O, C-C and respectively.

3) The IR Spectra of ocimum sanctum were obtained on a FTIR4100. Table 8.6 shows peaks observed at different wave numbers and the functional group associated with these peaks. These were identical with the reported values. Hence the samples were confirmed by their FTIR analysis. In this, N-H stretch =3356.25cm−1, aldehyde =2854.74cm−1, isothiocynate= 2044.61cm−1, aromatic C=C = 1458.23cm− &, -OH alcohol=3541.42cm−1 groups are present. All the characteristics peaks of *O.Sanctum* were present in the spectrum of drug & physical mixture, indicating compatibility between drug+ polymers. The spectrum confirmed that there is no significant change in absorption band, hence no interaction between them However, some additional peaks were observed with physical mixtures, which could be due to presence of polymer.

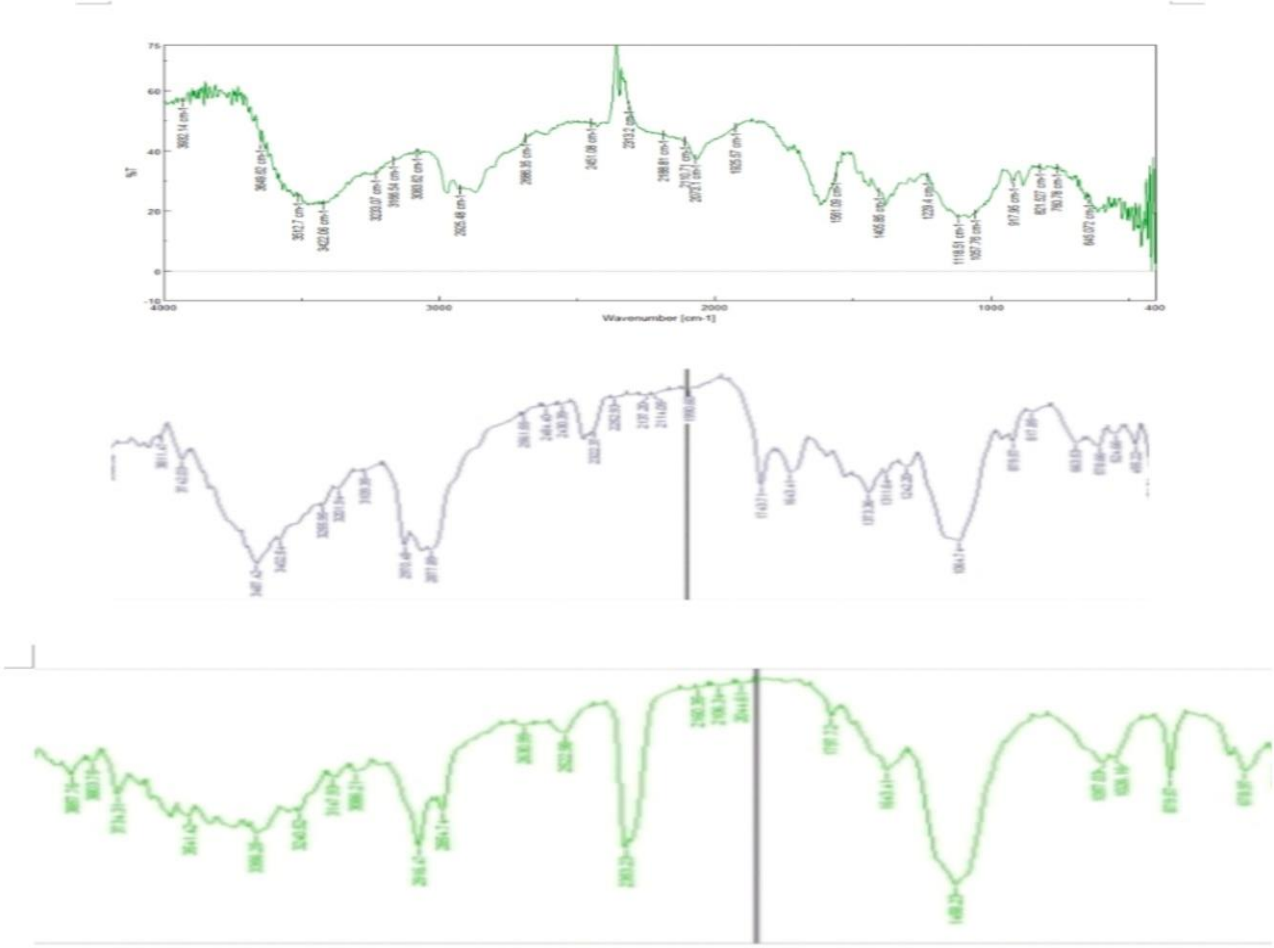
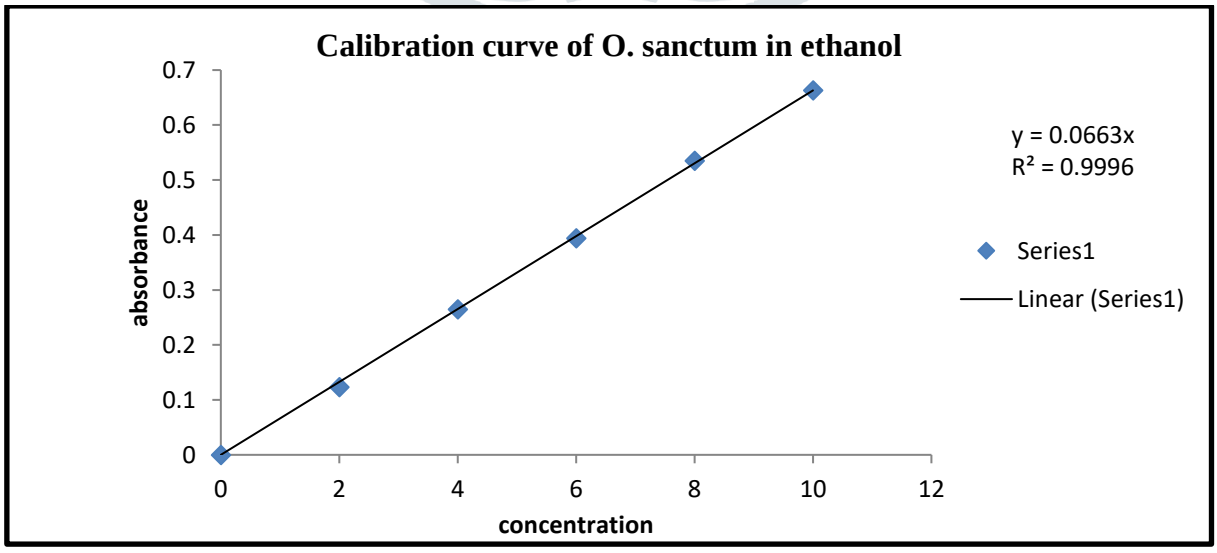


Fig 1: IR Spectra of 1) ethyl cellulose 2) physical mixture (drug+excipents) 3) ocimum sanctum extract

3. UV visible spectroscopy :

The UV spectrum λmax of *ocimum sanctum* extract in Ethanol was found to be 284 nm. The calibration curve of *ocimum sanctum* extract in Ethanol was found to be linear over the range of 2-10 µg/ml as shown in fig. (R^2 = 0.999) Hence, the calibration curve of *ocimum sanctum* extract extract followed Beer's – Lambert law over this range.



4. Particle size (µm):.

The particle size was determined at room temperature by using the Motic electron microscope. In the present work, the paricle size of ocimum sanctum microsponges is in the range of 41.86 µm to 110.93 µm. Formulation F1 exhibited minimum particle size of 41.86 µm. when both ethyl cellulose and PVA were at low levels and converse was also true, when both were at high levels (F9) shows particle size 110.93 µm. At given level of PVA when amount of ethyl cellulose incorporated is increase, particle size was found increased. Same changes were also observed in the concentration of PVA. The increase in particle size is to viscous organic phase produced at higher concentrations of ethyl cellulose, which results in larger sized emulsion droplets and larger micro porous colloidal particles.

5. Entrapment efficiency (%):

Drug: polymer ratio and amount of pore inducers affect the entrapment efficiency. An increase in the drug-polymer ratio could result in an increase in EE. The reason for increase in EE is the reduced diffusion rate of drug solution from concentrated polymeric solutions into the external phase. This gives droplet formation more time, resulting in higher microsponge yield and entrapment efficiency.

6. Production yield (%):

The production of ocimum sanctum was significantly impacted by drug: polymer proportion and concentration of the surfactant. Increase the drug: polymer ratio resulted into increased production yield, when drug: polymer ratio was 1:1 (F1) the production yield was very low, i.e. 46% while for optimized batch (F5) it was 55.30%. As the concentration of surfactant increased from 0.25% to 0.75% (F9) yield was noticeably reduced to 35.40%. The production yield was drastically reduced from the formulations F6-F9 due to high concentration of surfactant the reason for the less production may be due to the development of unreasonable foam.

Table no.2: particle size, entrapment efficiency, production yield of all the formulations.

Batch code	Particle size (µm)	Entrapment efficiency (%)	Production yield (%)
F1	41.863 µm	41.22%	46%
F2	59.703 µm	45.24%	51%
F3	70.462 µm	52.12%	53.30%
F4	75.357 µm	58.10%	46.66%
F5	88.462 µm	69.12%	55.30%
F6	109.803 µm	63.15%	52.20%
F7	99.449 µm	60.10%	40%
F8	102.967 µm	59.31%	30.1%
F9	110.933 µm	61.15%	35.40%

7. Scanning electron microscopy (SEM) :

Scanning electron microscopy of the optimized batch of the ocimum sanctum loaded microsponges as shown in fig.2 .It is clear from the figure that microsponges have spherical shape and spongy appreance.

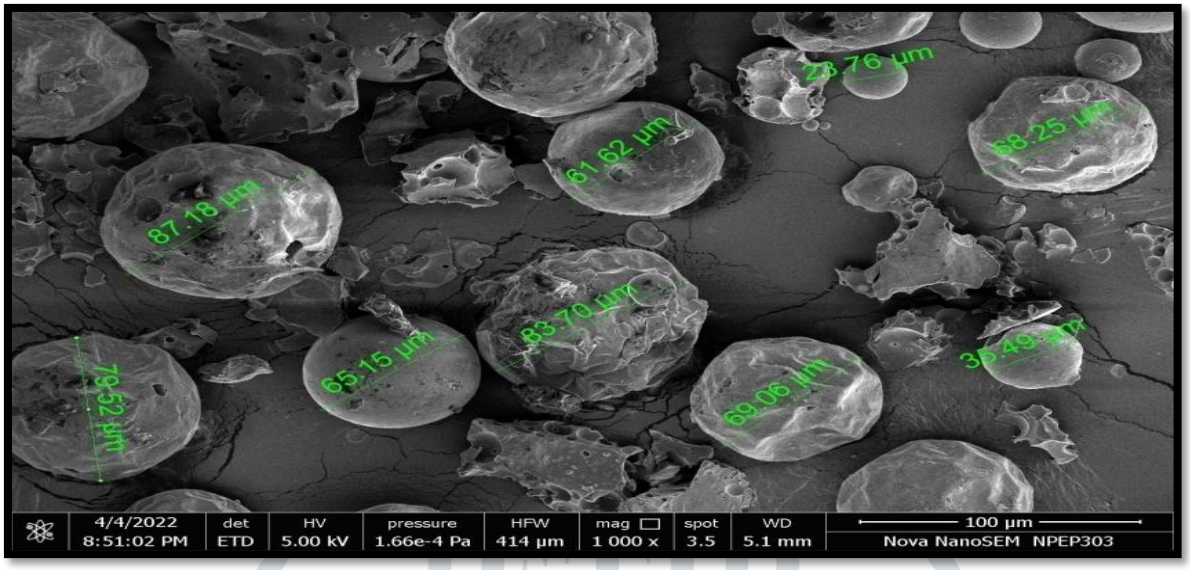


Fig 2: SEM of optimized microsponge formulation (F5)

8. Evaluation parameters of Ocimum sanctum loaded microsponge based gel :

(1) Physical examination :

All the Carbopol 934 gel formulations were light green colored, semisolid with smooth consistency and homogeneity. It was washable.

(2) pH :

pH of all the gel formulations was within an acceptable range 5.2 to 5.4

(3) viscosity :

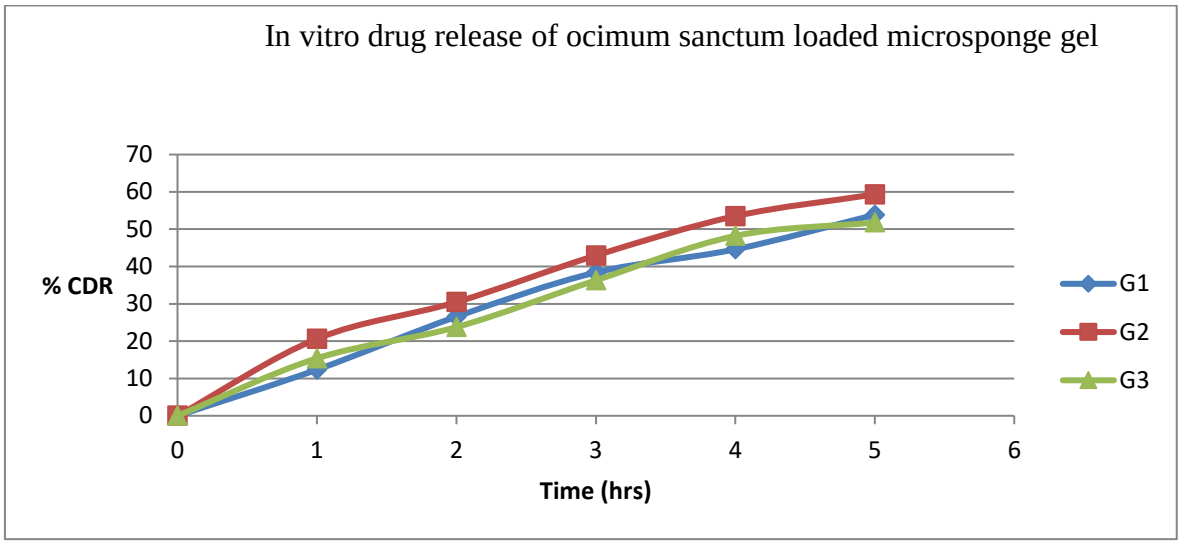
Viscosity of gel was found in the range of 39890 cps to 42045 cps.

(4) Spredability :

Spredability of gel was in range 7.2 to 9.2 gm/cm/sec.

(5) *In vitro* drug release (%) :

% drugs release of optimized ocimum sanctum microsponge loaded gel was carried out by using Franz cell diffusion apparatus by using egg membrane. In vitro drug release was found in the range of 59.34±0.2 to 51.8± 0.1 at 5 hrs for G2 and G1 . amomg all the gels, G2 formulation is optimised on the basis of pH, viscosity, spredability and drug release 59.34±0.2 within 5 hrs.



(6) *In-vitro* antifungal activity :

Table no.3 : Efficacy against *candida albicans* of ocimum sanctum loaded microspong gel and ocimum sanctum plain gel (Interpreted as zone of inhibition in mm)

Sr.No	Zone of inhibition (mm)	
	sample	Zone of inhibition (mm)
1	Ocimum sanctum ethanolic extract	8 mm
2	Plain gel	10 mm
3	Ocimum sanctum loaded microsponge based gel	13 mm

Fig 5: Comparative antifungal activity of ocimum sanctum extract, ocimum sanctum gel and ocimum sanctum loaded microsponge gel

Ocimum sanctum microsponge loaded gel having large zone of inhibition as compared to plain ocimum sanctum gel. With the help of this antifungal activity concluded that, microsponge loaded gel has better antifungal activity.

CONCLUSION:

The current research demonstrates a simple, quick and cost effective approach for producing ocimum sanctum loaded microsponges by quasi emulsion solvent diffusion method. Drug loaded microsponges were successfully prepared by using ethyl cellulose and polyvinyl alcohol. The study concluded that when the concentration of polymer and PVA increases PS and EE is increases. It was observed that when concentration of Carbopol 934 rises viscosity increases & spredability decreases. So, the gel with 1% Carbopol 934 (G2) gel selected as optimized formulation based on good results compared with others. Hence, gel incorporated with ocimum sanctum loaded microsponges can be used as an appropriate formulation for the treatment of candidiasis.

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