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ANTIMICROBIAL POTENTIAL OF HYDROGEL INCORPORATED WITH PLGA NANOPARTICLES OF *CROSSANDRA INFUNDIBULIFORMIS*

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ABSTRACT

Objective: Present study is aimed at formulation of Hydrogel containing Poly Lactic Glycolic Acid (PLGA) nanoparticles incorporated with ethanolic extract of *Crossandra infundibuliformis* (EECI) and investigate the efficacy of hydrogel nanoparticles as a carrier of antimicrobial constituents.

Methods: Poly Lactic Glycolic Acid (PLGA) nanoparticles containing ethanolic extract of *Crossandra infundibuliformis* (EECI) were synthesized by an emulsion-evaporation method and their physicochemical properties were studied. Polymeric PLGA nanoparticles were then incorporated into gel matrix, using Hydroxy Propyl Methyl Cellulose (HPMC K_4M) as a base. The antibacterial activity of nanoparticulated hydrogel formulations were evaluated by agar well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa.*

Results: Nanoparticulate hydrogel formulations exhibited high viscosity, neutral pH with good spreadability which is appropriate for transdermal application as well as showed prolonged drug release from optimized formulation up to 24 h. Nanoparticulate hydrogel formulations were effective inhibitors of all the micro-organisms with more promising activity against *Staphylococcus aureus*.

Conclusion: Nanoparticulate hydrogel formulation can be used as a feasible alternative to conventional formulations of *Crossandra infundibuliformis* extract with advanced permeation characteristics of antimicrobial constituents for transdermal application.

Keywords: *Crossandra infundibuliformis,* Nanoparticles, Hydrogel, Antimicrobial activity

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INTRODUCTION

In recent years, encapsulation of antimicrobial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs. Many antimicrobial drugs are difficult to administer because of their low water-solubility, cytotoxicity to healthy tissues, and rapid degradation and clearance in the bloodstream. Their antimicrobial activities against intracellular microbes are also severely limited by poor membrane transport ability. Extensive studies have demonstrated that nanoparticles such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, and dendrimers are able to overcome these issues and facilitate antimicrobial delivery to microbial infection sites [1].

Polymeric nanoparticles are made from biodegradable and biocompatible polymers, represent an option for controlled drug delivery and are promising formulation used for drug delivery systems, because they can be targeted whereas hydrogel nanoparticles, which are currently being widely explored for the delivery of hydrophilic as well as hydrophobic actives, comprise particles of polymeric mesh with a high capacity to imbibe water or biological fluids due to the presence of hydrophilic groups on their surface. They offer unique characteristics of swelling in water, instead of being dissolved in the surrounding aqueous medium, due to the crosslinks present in the polymeric structure [2].

Crossandra infundibuliformis belonging to family Acanthaceae is observed to show several medicinal properties and especially, its leaf extract was reported to have the antioxidant, antibacterial, antifungal, anticandidal and larvicidal activity [3-6]. These activites may be due to the presence of phytoconstituents such as carbohydrates, flavanoids, alkaloids, steroids, tannins, terpenoids, Saponins which was reported in the extracts of *Crossandra infundibuliformis* leaves from the phytochemical analysis [7]. Leaf extract was also found to effective topically for wound healing [8]. Considering the above facts, present study was done to exploit the size and hydrophilic nature of the formulated nanocarriers to enhance the antimicrobial effect.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *Crossandra infundibuliformis* were collected from local area of Bantwal Taluk and authenticated by Prof (Dr) Nagalakhamma St. Aloysius College, Mangalore. Plant material was washed under running tap water to remove adhering dust, dried under shade and powdered. Powdered leaf drug was extracted by cold maceration method using 95% ethanol as a solvent for 7 d. After the extraction solution was filtered and the filtrate was evaporated to dryness and the percentage yield was calculated.

Calibration of *Crossandra infundibuliformis* extract by UV-visible spectrophotometer

The ethanolic extract was dissolved in small amount of ethanol, followed by addition of phosphate buffer of pH 6.8 and then suitably diluted. The solutions were scanned for its specific UV-visible range of absorbance maxima. Then the absorbance of the different serial diluted samples was measured at the λ max, and a standard calibration curve was plotted with concentration against absorbance.

Nanoparticle synthesis

For the synthesis of polymeric nanoparticles, PLGA (50:50) was procured from Sigma Aldrich, Bangalore Three nanoparticle formulations (N1, N2, N3) with different extract/polymer ratios were synthesized by emulsion-solvent evaporation method [9]. During the process, the organic phase was prepared by dissolving accurately weighed PLGA and plant extract in dimethyl sulphoxide (DMSO) as organic solvent. The organic phase was then added dropwise at the rate of 1 ml/min into an aqueous phase containing surfactant i. e Poly Vinyl Alcohol (PVA-0.5%). The nanoparticles suspension was kept under continuous stirring at 300 rpm for 3 h at 30 °C to allow the complete evaporation of DMSO, leaving behind the colloidal suspension of PLGA nanoparticles containing plant extract in the aqueous phase. The colloidal nanosuspension was centrifuged at 12,000 rpm (Remi, Mumbai, India) for 30 min at 4 °C to get the final

nanoparticulate containing pellet as encapsulated plant extract. The pellet was washed with deionized water twice to remove the un-entrapped drug from the surface of nanoparticles. The obtained nanoparticles loaded suspensions were stored at 4°C until further use. Free nanoparticles were synthesized with the same protocol without extract. Formulation ingredients of nanoparticles are given in table 1.

Table 1: Composition of polymeric nanoparticle

Formulations	Drug (mg)	Polymer (mg)	Surfactant (%)
N1	25	50	0.5
N2	25	100	0.5
N3	25	200	0.5

Characterization of polymeric nanoparticles

Surface morphology

Scanning electron microscopy SEM (JEOL, JSM 50A, Tokyo, Japan) was performed to evaluate the shape and the surface morphology of nanoparticles. Transmission Electron Microscopy (TEM) was also used to evaluate nanoparticle morphology.

Particle size analysis, polydispersity index, and zeta potential measurements

Average particle size (z-average) and Polydispersity index (PDI) of the developed nanoparticles were determined by laser dynamic light scattering using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK). The PDI value indicates the particle size distribution of nanoparticles in a given sample.

Zeta potential indicates the surface charge on the particles and was measured to determine the stability of nanoparticles in the suspension. Zeta potential data of nanoparticles loaded with extract was collected through electrophoretic light scattering using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK) [11].

Entrapment efficiency of the polymeric herbal nanoparticle

The nanoparticle suspension formulated with the extract and polymer was ultra-centrifuged at 18,000 rpm for 30 min in a cooling centrifuge apparatus and then the supernatant solution was diluted suitably to measure the absorbance, from which the concentration of drug in the supernatant was calculated using the standard calibration data [12]. The entrapment efficiency of the extract in the polymeric nanoparticles was calculated using the formula,

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total amount of Drug added- amount of Drug is supernatant}}{\text{Totan amount of Drug added}} \times 100$$

Based on the results of particle size analysis, PDI and entrapment efficiency, an optimised formulation of nanoparticles was chosen and it was subjected for the formulation of nanoparticles

Formulation of nanoparticulate hydrogel

The optimized polymer nanoparticle (N2) was incorporated in the hydrogel matrix. HPMC K4M was selected as gel matrix base. HPMC K4M was obtained from Yarrow Chem products, Mumbai. Various ingredients used in the formulations are shown in table 2. Required quantity of gelling agent was weighed and dispersed in a small quantity of distilled water to form a homogeneous dispersion. Prepared nanoparticle formulation was added to the above solution to obtain hydrogel formulation HN1, HN2 and HN3. Other excipients were also added with stirring. The pH of this gel preparation was maintained 6.5±05 by using Triethanolamine and stored in well closed.

Table 2: Composition of hydrogel nanoparticles

Ingredients	HN1	HN2	HN3
N1	10 mg	10 mg	10 mg
Carbopol 934	500 mg	500 mg	250 mg
HPMC K4M	250 mg	500 mg	500 mg
Glycerine	2 ml	2 ml	2 ml
Sodium benzoate	100 mg	100 mg	100
Triethanolamine	q. s	q. s	q. s
Distilled water	q. s	q. s	q. s

Antimicrobial screening of extracts

Ethanolic extract of *Crossandra infundibuliformis* was evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activity was determined by the agar well diffusion method using Muller Hinton agar media. Each petri dish containing Muller-Hinton agar medium was inoculated with one bacterial culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. Wells were made on the agar surface with 6 mm cork borer. The first cup was filled with Ciprofloxacin, the second was filled with dimethyl formamide and other is filled with extracts (50 mg/ml) using the sterile syringe. The plates were incubated at 37±2 °C for 24 h. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter [13]. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

***In vitro* drug release study**

Release study of hydrogel nanoparticles HGN1, HGN2, HGN3 was conducted to determine the release of the extract constituents from the formulations by dissolution method. *In vitro* release of the drug across the dialysis bag (12 Kda, Hi Media) soaked in deionized water for 12 h before use was performed by using diffusion cell (containing 1 ml of sample) and 80 ml of phosphate buffer pH 6.8 as the dissolution medium (n=6). The dissolution medium was maintained at 37±0.5 °C and the medium was stirred at 100 rpm with the help of small teflon coated magnetic bead. Aliquots of the medium were withdrawn at a suitable time interval and were replaced with the same volume of fresh medium to maintain the sink condition. These samples were filtered through 0.45 µm membrane filter and the collected samples were analyzed using UV-visible spectrophotometer at the λmax of 203 nm. The concentration of extract constituents was calculated by comparing the concentration to the standard calibration curve.

Characterization of hydrogel formulations

Determination of drug content

The amount of drug contained in the prepared hydrogel was determined by dissolving 100 mg of prepared hydrogel in acetone and diluted using phosphate buffer of pH 6.8. This mixture was analysed UV spectrophotometer at 203 nm against phosphate buffer as blank.

pH determination

The pH of the formulation was determined at ambient temperature with digital pH meter (digital pH meter, 335, systronics, Noroda, Ahmedabad).14 since the formulation was a topical formulation to be applied to the skin, therefore pH measurement was essential to ensure non-irritating nature of the formulation [13].

Determination of spreadability

The spreadability of prepared hydrogel formulation was determined 48 h after preparation by measuring the spreading diameter of formulation between the two glass plates after 1 min. A weight of 500 mg of hydrogel was placed within a circle of diameter 1 cm premarked on a glass plate over which a second glass plate was placed. The increase in diameter as a consequence of weights added leading to the spreading of gel was noted [14].

Measurement of viscosity

The viscosity of the prepared formulations was determined at different angular velocities at 32.0±0.1 °C using spindle number 4 (Brookfield DV-II+Pro viscometer) [15].

RESULTS

Calibration of the crude extract

Ethanolic extract of *Crossandra infundibuliformis* were greenish in colour and percentage yield was found to be 14% respectively. The crude ethanolic extract of *Crossandra infundibuliformis* was dissolved in the little amount of ethanol and diluted with phosphate buffer of pH 6.8 and scanned in a UV-spectrophotometer have shown the maximum absorbance wavelength at 203 nm. The serially diluted samples of the extract exhibited the absorbance value in the linearity range and their regression was found to be 0.998 (fig. 1).

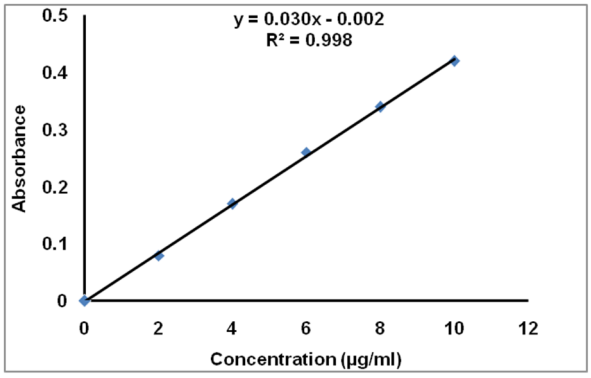

Fig. 1: Calibration curve of ethanolic extract of *Crossandra infundibuliformis*

Characterization of polymeric nanoparticles

The results of entrapment, size and charge of the nanoparticles are shown in table 3, where N1, N2, N3 are nanoparticles of *Crossandra infundibuliformis* ethanolic extract with different concentration of PLGA respectively.

Table 3: Physicochemical characterization of nanoparticles loaded with extract of *crossandra infundibuliformis*

Formulations	Particle size	PDI	Zeta Potential	Entrapment Efficiency
N1	143.9	0.235	-2.08	68.5
N2	283.8	0.267	-3.24	65.3
N3	321.8	0.299	-3.58	62.9

All values are expressed as mean±SD (n=3); N1: nanoparticles with 50 mg PLGA, N2: nanoparticles with 100 mg of PLGA, N3: nanoparticles with 200 mg PLGA

Scanning electron microscopy (SEM)

Shape and surface morphology of the nanoparticles in optimized formulation N1 was studied using SEM. The SEM study reveals that polymeric nanoparticles were spherical in shape with an average particle size around 143.9 nm as shown in fig. 2.

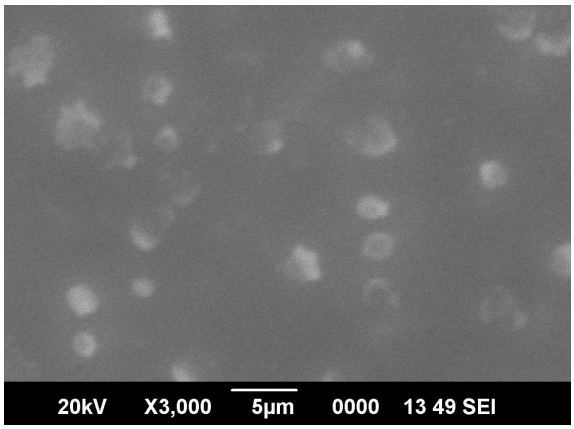

Fig. 2: SEM image of optimized PLGA nanoparticle formulation (N1)

Transmission electron microscopy (TEM)

The transmission electron microscope revealed a positive image in which nanoparticles appeared dark with bright surroundings as shown in fig. 3. The particle size of sample was less than 1000 nm

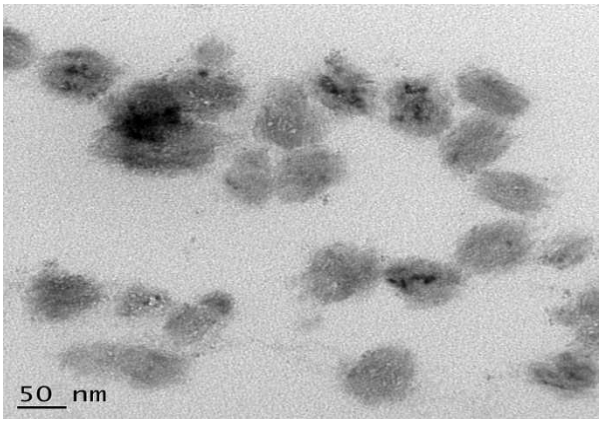

Fig. 3: TEM image of optimized PLGA nanoparticle formulation (N1)

Based on results of particle size and entrapment efficiency, nanoparticle formulation N1 containing *Crossandra infundibuliformis* ethanolic extract with 50 mg of PLGA polymer was selected as optimised formulation as it has nanosize range (<200 nm) particles with low PDI and sufficient entrapment efficiency.

Formulation and characterisation of nanoparticulate hydrogel formulation

Hydrogel formulations HN1, HN2, HG3 were prepared by incorporating optimised polymeric nanoparticle formulation (N1) to varying ratios of HPMC K4M: carbopol 934 hydrogel forming matrix 1:2, 1:1 and 2:1 respectively. Prepared hydrogel were subjected for

determination of pH, spreadability, viscosity and drug content and results of which is shown in table 4.

The drug content of nanoparticulate hydrogel formulation was in the range of 96.6±0.38% to 97.8±0.23%. The pH values of different nanoparticulate hydrogel formulations were found to be in a range of 6.6–6.8 (nearly neutral). The spreadability of the all formulations exhibited slip and drag phenomenon with higher diameters. Viscosity of hydrogel formulation decrease as the ratio of carbopol: HPMC decreases.

Table 4: Characterization of hydrogel nanoparticles

Formulations	Drug content	pH	Spreadability (cm)	Viscosity (m. PaS)
HN1	97.8±0.23	6.7±0.2	6.0±0.32	4312±0.21
HN2	96.4±0.21	6.6±0.3	5.5±0.22	4325±0.18
HN3	96.6±0.38	6.7±0.1	5.8±0.42	4350±0.16

All the values are expressed as mean±SD (n= 3), HN1, HN2, HG3: Hydrogel formulations with HPMC K4M: carbopol 934 in the ratio 1:2, 1:1 and 2:1.

Release studies from nanoparticulate hydrogel formulation

Drug release from hydrogel formulation HGN3 was found to be the highest of 89.01% whereas it was found to decrease to 75.42% and 44.67% respectively from formulation HGN2 and HGN1 at the end of 24h.

The drug release of formulation HGN1 was found to be 44.67 % due to the presence of large concentration of carbopol 934, on the other hand, formulation HGN2 and HGN3 shown an increased release of 75.42% and 89.01% respectively. On the basis of drug release study, formulation HGN3 was found to be optimized prepared.

Antibacterial activity

Antibacterial activity was investigated against four bacterial strains i.e *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* and among these *S. aurei* was most susceptible organism with inhibition zones ranging from 13-27 mm (table 5).

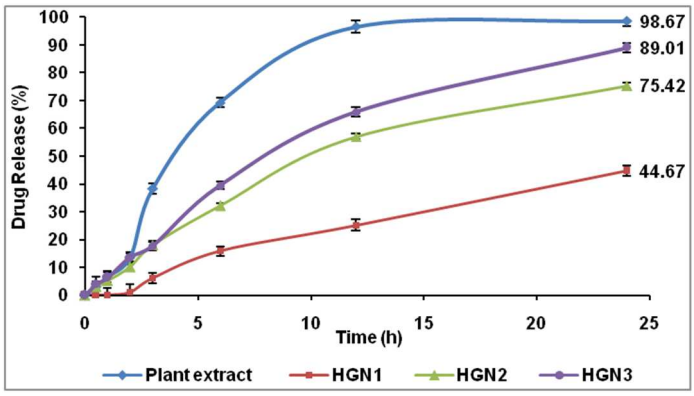

Fig. 4: *In vitro* drug release profile of nanoparticulate hydrogel loaded with extract of *Crossandra infundibuliformis,* HN1,HN2, HN3: Hydrogel formulations with HPMC K4M: carbopol 934 in the ratio 1:2, 1:1 and 2:1

Table 5: Zone of inhibition against bacterial strains

Samples	Zone of Inhibition (mm)			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
EECI	28±0.48	12±0.31	13±0.33	16±0.21
PLGA nanoparticles(N1)	27±0.41	12±0.21	12±0.24	16±0.24
Hydrogel(HN1)	27±0.51	11±0.23	12±0.31	16±0.23
Ciprofloxacin(Standard)	29±0.21	22±0.32	17±0.26	19±0.27

All the values are expressed as mean±SD (n= 3).

DISCUSSION

Hydrogels comprise thermodynamically balanced ‘‘solid-like solution’’ of polymer and water where equilibrium exists between the swelling of the hydrophilic polymer strands and the elastic forces of tie-points or junctions of the polymer network [16]. Hydrogel nanoparticles offer the combined advantages of both, the hydrogels and the nanoparticulate drug delivery systems which include hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility along with the prolonged circulation time and active/passive targeting ability of the nanoparticles [17]. The present investigation attempted the exploitation of these pharmaceutical advantages of the hydrogel nanoparticles to enable an enhanced absorption by the virtue of the hydrophilic surface of the carrier system.

In this study, initially *Crossandra infundibuliformis* extract was loaded into PLGA polymer which is biodegradable, biocompatible and its sub-products of degradation are non-toxic in superficial tissues. Prepared PLGA nanoparticles loaded with *Crossandra infundibuliformis* extract were spherical in shape, are of highly homogenous formulations with nanosize range and had better entrapment efficiency which was indicated by the SEM/TEM, particle size analysis and drug entrapment efficiency value. The low PDI values indicated that nanoparticles size was uniform within each formulation and found to be stable which was indicated by the zeta potential value. The stability of these nanoparticles could be related to the fact that NPs with high zeta potential (in modulus) produces repulsion among the NPs avoiding NP aggregation. Due to good physicochemical properties and capacity to encapsulate drugs,

prepared polymeric NP was used as nanocarrier for the phyto-constituents in the extract of *Crossandra infundibuliformis*.

Prepared nanoparticles were incorporated into a hydrogel which acts as a carrier for transdermal delivery of phytoconstituents in the extract due to its high solubilizing ability and permeation enhancing properties. The hydrogel formulations were subjected to drug content determination which showed that drug loss during formulation occurred within the limits. The pH was near to pH of the skin which revealed non-irritating nature of the formulation. The spreadability of the all formulations exhibited slip and drag phenomenon with higher diameters.

Hydrogels are appealing for numerous biomedical applications because of the material's ability to serve as a reservoir that releases its contents along a predetermined timeframe. The hydrogel nanoparticle formulations showed the release of phytoconstituents in the sustained manner from hydrogel, due to the presence of the sustained release polymer carbopol and HPMC. As the concentration of HPMC increased the drug release was found to increase due to its hydrophilic nature**.** On the basis of drug release study, formulation HGN3 was found to be optimized formulation. The hydrogels showed good antibacterial activity against *S. aureus* when compared with other micro-organisms.

CONCLUSION

Hydrogel formulation containing PLGA nanoparticles loaded with *Crossandra infundibuliformis* was successfully formulated and showed the sustained release of active constituents with enhanced permeation, spreadability which makes it good carrier for transdermal delivery of antimicrobial constituents in the extract. Hydrogel nanoparticles of *Crossandra infundibuliformis* in an aqueous gel base can be used as an appropriate formulation for the antimicrobial activity.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

No conflict of interest

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