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Fabrication of microspheres and characterization of antimicrobial and anti-inflammatory activity isolated fraction from total alcoholic extract of *Cassia Fistula* (Linn.) in carrageenan-Type-IV induced inflammatory rats

Beldar Shamshad Bee[1*], Rakesh Kumar Jat[1] and Sufiyan Ahmad[2]

[1]Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India;
[2]Gangamai College of Pharmacy, Nagaon, Dhule, Maharashtra, India

E-mail/Orcid Id:

BS, shamassshama@gmail.com, https://orcid.org/0009-0000-2246-5472;
RJ, phd@jjtu.ac.in, https://orcid.org/0000-0001-7720-0308; *SA,* sufipharm1712@gmail.com, https://orcid.org/0000-0003-4131-4025

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Abstract: The *Cassia fistula* Linn is known as the golden shower tree. It's also referred to as a "disease killer" in Ayurvedic medicine. The current study investigates the effect and estimate of antimicrobial and Anti-inflammatory activity guided fraction isolated from alcoholic extract of *Cassia fistula* (Linn.) root on carrageenan-Type IV induced Inflammatory rats for using Column fraction of alcoholic extract of root of *Cassia fistula* (Linn.) An in vivo carrageenan-induced paw edoema model was used to confirm further the anti-inflammatory effect of the fraction aloe-emodin and physcion-loaded microspheres. This study was designed first time for the isolation of alcoholic fraction of aloe emodin and Physcion by root extract and screening of pharmacological activities of *Cassia fistula.* Aloe-emodin and Physcion fraction demonstrated antibacterial and antifungal effectiveness against the studied pathogens in a dose-dependent manner, according to anti-microbial activity. 50 mg/ml concentration of Physcion-containing microspheres and aloe emodin's antibacterial efficacy against tested pathogens. The anti-inflammatory activity of Aloe emodin and Physcion was found in an equal manner. Both Microsphere samples were found capable of controlling the inflammation till 6 hours. According to the findings of this research, the chosen plant extracts have an immediate anti-inflammatory effect. There are three stages during which carrageenan-induced inflammation mediators are released.

Introduction

The *Cassia fistula* Linnis popularly known as the golden shower tree. It is extensively utilized for its medical benefits, with its significant benefit being that it is a gentle laxative appropriate for children and pregnant women. Golden Shower Tree is called "disease killer" in Ayurvedic medicine. The fruit pulp of this plant is a moderate laxative. Along with heart disorders and digestive issues like acid reflux. Both the root and the blossoms can be used to treat fever. Conditions of the skin are alleviated by using the leaves and bark. The seeds have been exploited for their antibilious, aperitif, carminative, and laxative therapeutic characteristics. At the same time, the root has been utilised adenopathy, burning sensations, leprosy, skin problems, syphilis, and tubercular glands. The fruit treats stomach ache, constipation, fever, heart illness, leprosy, and erysipelas.

In contrast, the leaves and buds treat some diseases, such as rheumatism, ulcers, and erysipelas (Dama et al., 2011). Therefore, the therapeutic powers of this plant are present throughout the entire plant. The term "microparticulate" may be unfamiliar to some readers. However, microspheres are simply microscopic solid spheres with a diameter of 1-1000 m that are coated in a protective material such as a polymer, lipid, or biodegradable synthetic polymer or a modified natural product such as polysaccharides, gums, proteins, or lipids. For instance, albumin is one type of natural

***Corresponding Author:** shamassshama@gmail.com

polymer. Similarly, polyglycolic acid and its derivatives are included in the synthetic polymers (Zhu et al., 2023).

There are two categories of microparticles:

(1) Microcapsules with a unique capsule wall entirely enclosing them.

(2) A substance that is spread throughout the material matrix in the form of microspheres.

A crucial component of NDDSs, microspheres offer the capacity to deliver drugs in a regulated way. Due to its biodegradability, benign nature, minimal side effects, and superior therapeutic value, advancement in the herbal sector employing innovative technologies for drug administration or carriers is considerably boosted. Microspheres containing herbal substances or extracts can target the location of action with little toxicity, increasing bioavailability.

Toxicology is part of pharmacology that arranges the antagonistic impact of bioactive substances on living life forms. To set up the wellbeing and effectiveness of new medication, toxicological studies are key examinations in animals like mice, rodents, and so forth. No medication substance is utilized clinically without its research centre security appraisal at the preclinical stage. Toxicological studies help decide whether new medication should be received for clinical utilization. Enhancing the pharmacological action of Aloe-emodin and Physcion with the features of micro formulation with long-lasting activity may be created (Mane et al., 2023). To do this, a fraction of aloe-emodin and physcion-loaded microspheres were made experimentally and subsequently characterised. After conducting an analysis of the physico-chemical properties, this study examined how the quantity of aloe-emodin and physcion used in the formulation affected these features. The in-vivo anti-inflammatory properties of the substances were evaluated in comparison to carrageenan-Type-IV. Neutrophils, the creation of free radicals by neutrophils, and the release of other mediators produced by neutrophils are all linked to the inflammation triggered by carrageenan. Inhibition of carrageenan-induced inflammation in rats is a well-established paradigm for testing anti-inflammatory medicines. This model has been used extensively to evaluate the anti-inflammatory efficacy of various natural treatments. Within an hour of carrageenan-induced inflammation, the initial phase of carrageenan-induced edoema formation begins, and it is linked to the release of cytoplasmic enzymes serotonin, from mast cells. Increased prostaglandin release in the inflammatory region mediates the second phase, with kinins providing continuity between the two (Zahid et al., 2023).

Materials and methods

Materials

Aloe-emodin and physcion isolated fraction from the total alcoholic extract of *Cassia fistula* (Linn.) Diclofenac, Albino wistar rats weighing 180 ± 20 gm, Nutrient broth, H.V and L.V grade PGA,1% carrageenan-Type-IV. The investigation only employed analytical-grade solvents and materials. The experiment was conducted with deionized, double-distilled water (Zlatić and Stanković, 2020).

Methods

Preparation of microspheres

Slight modifications were made for microspheres preparation and Labultima LV 222 Advanced, (Mumbai, India) spray dryer was used. The formulation consisted of different drug to polymer ratio concentrations for each batch. The fraction of Aloe-emodin and physcion was dispersed in the prepared polymer solution in distilled water separately. The feed solution was continuously stirred and sprayed in a drying chamber with a flow rate 5 mL/min (inlet temp 120ºC, outlet temp 60ºC). Microspheres were collected from cyclone vessels (Yang et al., 2023).

Physicochemical Evaluation of the microspheres

Production yield

Percentage production yields were calculated for different batches of microspheres by comparing the weight of the dried product to the total weight of the drug and polymer used in their preparation, as shown in the equation below.

$$\%\ \text{Production Yield} = \frac{A}{B} \times 100$$

Where A= Weight amount of final spray-dried microspheres

B = Dry starting material (EPS and PGA) used in the formulation.

Drug content and incorporation efficiency

Accurately weighed microspheres of 10 mg were soaked in methanol and kept all night. The UV-Spectrophotometer (Shimadzu, 1700) determined drug content at 390 nm. Drug content (D.C. %) and Incorporation efficiency (I.E., %) was determined using the following equations.

$$\text{Drug content} = \frac{\text{Actual amount of EPS in microsphers (mg)}}{\text{Amount of microspheres (mg)}} \times 100$$

$$\text{I.E. } (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where W_1= Amount of drug loaded in formulation

W_2= Amount of drug in supernatant.

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Differential Scanning Calorimetry (DSC)

The thermal examination was investigated for pure drug (EPS), polymer (PGA) and drug-loaded microspheres using DSC (Mettler Toledo). Sample weighted 2 mg was sealed in aluminum pan. Various sample data were recorded in the heating range of 40-300º C with 20 mL per min nitrogen flow.

Scanning electron microscopy

SEM images of selected formulation batches were observed by SEM (JSM 6390, JEOL). Microspheres were first sprinkled onto aluminium stubs and placed in a vacuum chamber. Images were taken at an increase of rate voltage of 20 kV with a magnification of X3500 to X7000.

Size analysis

It is performed at an angle of X-45 using a microscope. Once dried microspheres have been rehydrated in distilled water and placed in a glass slide, a stage micrometre can count several divisions in a calibrated eyepiece. Find the particle size distribution.

Melting point

Remove the covering material from a sample of microspheres by grinding them and then determine their melting point.

Infrared spectroscopy

Mix around 300 milligrams of dry, finely powdered potassium bromide IR with about 1 milligramme of the microspheres and triturate. Completely grind the ingredients, evenly distribute them in the die, and then crush them under vacuum at a pressure of around 800 Mpa. Put the resulting disc into the spectrophotometer's holder to get IR readings.

XRD study

Diffraction pattern of EPS (pure drug), EPS loaded microspheres with low and high viscosity grades were studied by Brucker AXS D8 Advance, carried out at voltage 40 kV and current of 35 mA.

***In-vitro* drug release**

The modified Franz's diffusion cell was used to analyse the drug release profile. The dialysis membrane (molecular weight 12000) was equilibrated in phosphate buffer before the study. Sink condition was maintained at a temperature 37±0.5^{0}C and stirred at rpm 100. About 10 mg microspheres were placed above donor compartment. A diffusion membrane separated the donor and receiver compartments. The diffusion medium was a phosphate buffer solution (pH=6.4). The same volume of diffusion medium was replaced after each sample to retain sink condition. The number of samples was analyzed by UV-Spectrophotometer at $\lambda_{max}$of 434 and 286 nm. The *In-vitro* diffusion release study was done in triplicate.

Drug release kinetic

Several kinetic models, including the Zero, First Order, and Higuchi model, were applied to study of drug release from microspheres. See below for a breakdown of the models.

Zero Order

$$C = K_0 t$$

Where K_0 = rate constant expressed in units of concentration/time, t is time in minutes. When plotting concentration vs. time, a straight line with a slope of K_0 would pass through zero and one.

First Order

$$\text{Log} = \frac{\text{Log } C_0 - Kt}{2.303}$$

Higuchi model

$$Q_t = Kt^{\frac{1}{2}}$$

Mechanism of drug release

Korsmeyer-Peppas equation plotted as a function of log cumulative percentage of drug released v_s log t was used to assess the efficacy of microspheres' drug release mechanism.

$$\frac{M_t}{M_\infty} = Kt^n$$

n and K values of the release exponent were determined by the slope of a straight line (Dhadde et al., 2023).

Antimicrobial activity

The Disc Diffusion Method relies on analysing the growth response of different bacteria that come into contact with plants to identify their antimicrobial activity. *Cassia fistula* Linn. Microspheres will be used to test the alcoholic root extract fractions for antibacterial activity. To prevent contamination, the experiment was conducted inside a laminar air flow cabinet. A total of 25 ml of Nutrient Agar and Sabouraud Dextrose Agar were combined with 100 µl of a suspension of the test bacteria and fungus, each containing 1.0 x 105 colony forming units per millilitre. The inoculation media was carefully moved to Petri dishes where it could solidify in a germ-free environment. The screening was performed by soaking sterile paper discs in the extract at varying concentrations (10 µl/disc). For the bacterial and fungal strains, the usual discs of Ciprofloxacin and Fluconazole (10 µg/disc) were utilised as positive controls, while DMSO (10 µl/disc) was the negative control. This was then dried and pressed gently over the surface of the infected media agar plates before being incubated at 37°C for 24 hours for bacteria and 28°C for 72 hours for the fungal strain. Antibiotic zone reader's clear zone of inhibition was measured in millimetres, disc diameter included. Petri plates were autoclaved at 121^{0}C for 30

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minutes after determining the Zone of Inhibition diameter, and then disposed of properly (Frent et al., 2023).

Preparation of test samples

Microspheres of *Cassia fistula* were produced from alcoholic root extract fractions, in DMSO at 6.25, 12.5, 25, and 50 mg/ml concentrations.

Preparation of paper disc

Using a 6mm paper punching machine, we cut 6 mm discs using Whatman filter paper. Two hours at 160^0 Celsius in a hot air oven sterilised these.

Test microorganisms

The Microbial Type Culture Collection Centre (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, INDIA, supplied all of the pure cultures of the bacteria and fungi used in the experiments listed in Table 1. Table 2 lists many microbial characteristics. Sub-cultured on nutritional agar and Sabouraud dextrose agar (SDA) medium on a routine basis kept the bacterial and fungal cultures clean. We kept the pure cultures at 4 degrees centigrade.

***In vivo* anti-inflammatory activity**

Acute Carrageenan-induced paw edoema in rats served as a model for testing anti-inflammatory effects of the alcoholic extracts Fraction of Aloe-emodin and Physcion. Wistar albino rats were split into the eight different groups (n=4) described below. Underneath the plantar tissue of the right hind paw of rats, inflammation was generated by injecting 0.1 ml of freshly made 1% carrageenan aqueous suspension in normal saline. For comparison, animals in group 2 were given Diclofenac sodium (10 mg/kg) as the standard treatment, while those in group 1 received simply vehicle (1% CMC, p.o.). Fraction of Aloe-emodin and Physcion alcoholic extracts (100 and 200 mg/kg, p.o.) were given to animals in groups 3 through 8. Carrageenan (an edematogenic substance) was injected to induce paw oedema after 1 h of medication administration. The right paw of the rats was sub-planter injected with 0.1 ml of (1%, w/v) carrageenan in normal saline, causing acute inflammation. We used a Plethysmometer (UGO BASIL) to measure the volume of the paws at 30, 60, 120, and 180 minutes following carrageenan administration. Table 3 provides an in-depth description of the experiment.

Table 1. Micro-organisms used in antimicrobial activity

Serial No.	MTCC Code	Micro-organism	Type
1	MTCC 3165	*Bacillus subtilis*	Gram + ve bacteria
2	MTCC 3160	*Staphylococcus aureus*	Gram + ve bacteria
3	MTCC 614	*Escherichia coli*	Gram – ve bacteria
4	MTCC 424	*Pseudomonas aeruginosa*	Gram –ve bacteria
5	MTCC 227	*Candida albicans*	Fungus

Table 2. Culture conditions of micro-organisms

Sr. No.	Microorganism	Nutrient media	Incubation Time (hrs.)	Incubation Temperature
1	*Bacillus subtilis*	Nutrient agar	24	30ºC
2	*Escherichia coli*	Nutrient agar	24	37ºC
3	*Staphylococcus aureus*	Nutrient agar	24	37ºC
4	*Pseudomonas aeruginosa*	Nutrient agar	24	37ºC
5	*Candida albicans*	Sabouraud dextrose agar	72	28ºC

Anti-Inflammatory activity of isolated fraction (Aloe-emodin and Physcion) and containing microspheres

Experimental design

Adult male Albino Wistar rats weighing 180± 20 gm were used in the experiments after acclimating to the laboratory environment. Animals were fasted prior to dosing by withholding food overnight. They were given pellets and water from the tap to drink whenever they wanted.

Inhibition can be determined by solving the following equation.

$$\%\ inhibition = \frac{Ac - As}{Ac} X100$$

Where, As is the absorbance of sample. Ac is the absorbance of control, i.e., without sample

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Table 3. Experimental design anti-inflammatory activity of aloe-emodin and physcion

Groups	Treatment	Dose
1	Normal Control	-----
2	1% carrageenan + Diclofenac sodium	10 mg/kg, p.o.
3	1% carrageenan + Aloe-emodin 100	100 mg/kg, p.o.
4	1% carrageenan + Aloe-emodin 200	200 mg/kg, p.o.
5	1% carrageenan + Physcion 100	100 mg/kg, p.o.
6	1% carrageenan + Physcion 200	200 mg/kg, p.o.

Statistical analysis

All of the experiments were analysed statistically using one-way ANOVA. The ANOVA was double-checked with a post hoc Bonferroni t-test. The cutoff for significance was set at 0.05, statistically relevant. The data was summarised using a mean and standard deviation (SD) format (Shreya et al., 2023).

Table 4. Production yield of aloe-emodin and physcion containing microspheres

Sl. No.	Formulation code	Production yield (%±S. D.)
1	L.V. aloe emodin (F1)	42.63±2.20
2	H.V. aloe emodin (F2)	32.96±2.10
3	H.V. aloe emodin (F3)	36.35±2.30
4	L.V. Physcion (F1)	29.75±1.45
5	H.V. Physcion (F2)	28.44±0.74
6	H.V. Physcion (F3)	20.19±0.26

Table 5. Drug content and incorporation efficiency of aloe-emodin and physioncontaining microspheres

Sl. No.	Formulation code	Drug content (%±S.D.)	Incorporation efficiency (%±S.D.)
1	L.V. aloe emodin (F1)	12.68±0.07	75.15±0.79
2	H.V. aloe emodin (F2)	11.51±0.09	74.10±0.12
3	H.V. aloe emodin (F3)	9.25±0.12	75.19±0.10
4	L.V. Physcion (F1)	7.99±0.08	75.78±0.25
5	H.V. Physcion (F2)	5.99±0.08	77.63±0.34
6	H.V. Physcion (F3)	10.76±0.05	78.46±0.11

Results and Discussion

Characteristics of microspheres

Percent production yield was 20.19% - 42.63% for parastate microspheres. The relatively low values due to the attachment of material on the drying chamber, which can be affected by the properties of polymer and spray drying condition results, were tabulated in Table 4. The determination of drug content showed an increase in drug: polymer ratio decreased drug content. It was in the range of 5.99%- 12.68%. Aloe-emodin and Physcion-loaded microspheres were formulated with two grades of PGA. The microspheres with high viscosity grades relatively have higher drug incorporation efficiency than those with low viscosity grade as given in Table 5.

SEM is a valuable tool in characterizing microspheres and understanding their surface morphology. The information obtained from SEM images aids in optimizing the synthesis process, assessing the microspheres' quality, and tailor their properties for specific applications (He and Mu, 2023). Scanning Electron Microscopical image of formulation was observed to be oval to spherical in shape. Microspheres displayed smooth surfaces (Figure 1 and 2). XRD analysis of microspheres involves exposing the sample to X-rays, collecting the diffraction pattern, identifying the crystal phases present, and interpreting the data to gain insights into the material's structure and properties. It was determined by infrared research that Aloe-emodin and physcion, a proline-rich protein, interact with CRM (polyphenolic compound), and it was also used to examine how different polymers interact with one another. According to research, aloe-emodin and physcion may form aggregates and entrap solutes like Drugs or amino acids. Fraction aloe-emodin and physcion

Table 6. Particle size of aloe-emodin and H.V. physcion loaded microspheres

Sr. No.	Formulation code	Particle size (nm)
1	L.V. aloe emodin (F1)	1920
2	H.V. aloe emodin (F2)	214
3	H.V. aloe emodin (F3)	183
4	L.V. Physcion (F1)	2000
5	H.V. Physcion (F2)	693
6	H.V. Physcion (F3)	615

Table 7. Model fitting of the release profile from microspheres optimized batch

Batch	Kinetic models R^2			
	Zero order	First order	Higuchi	Best fit model
H.V. aloe emodin	0.858	0.584	0.904	Higuchi
	Koresmeyer Peppa's equation			
	R^2	K value	n value	Mechanism
H.V. aloe emodin	0.984	0.068	0.730	Non-fickian

loaded microspheres are shown in Figure 3.XRD studied the crystalline nature of microspheres with low and high-viscosity grade formulations. H.V. aloe emodin and physcion loaded microspheres showed high-intensity peaks at 25.45º and 22.87º. It proved spray drying cause encapsulation of sample in polymer matrix Figure 4 and 5.Particle size analysis is crucial for quality control, process optimization, and assessing the performance of microspheres in various applications, including drug delivery, coatings, fillers, and cosmetics, among others. The particle size of H.V. Aloe-emodin and H.V. physcion-loaded microspheres ranged from (2000 nm to 615 nm). The particle size smaller than 10 µm considered to be advantageous for Microparticulate delivery results were tabulated in Table 6. The aloe-emodin and physcion drug release profile from aloe-emodin and physcion-containing microspheres through dialysis membrane in PBS pH 6.4 as shown in Figures 6 and 7. Formulation containing high viscosity grade PGA showed a higher percentage of drug release than low viscosity grade containing formulation. Microspheres showed a sustained release effect at a constant rate for six hours.

The formulation containing L.V.F. gives a percentage of drug release of 62%. Compared to L.V.F. formulation H.V.F. formulation gives percent drug release till 90%. Since the drug release profile batch H.V.F. 2 batch gives more drug release. The data concluded that batch H.V.F. 2 as selected for further evaluation of drug release kinetic from aloe-emodin loaded microspheres release constant, was calculated from the slope of appropriate model and regression coefficient (R^2). Optimized batch H.V. aloe-emodin was best fitted to Higuchi model shown (R^2=0.904). The Korsmeyer Peppas equation indicated an excellent linearity of regression (R^2=0.984). Release constant 'n' was found to be (0.730), and the K value was

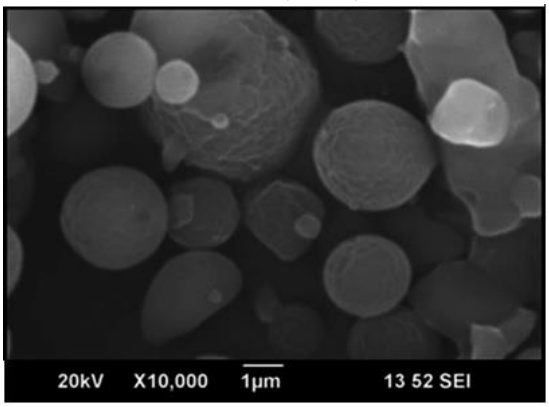

Figure 1. SEM image of microspheres of selected batch H.V. aloe-emodin

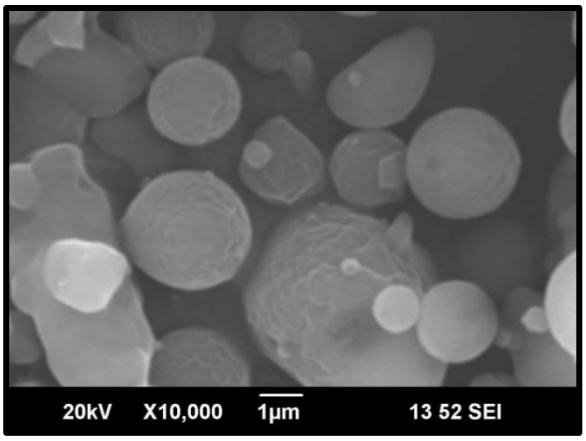

Figure 2. SEM image of microspheres of selected batch H.V. physcion

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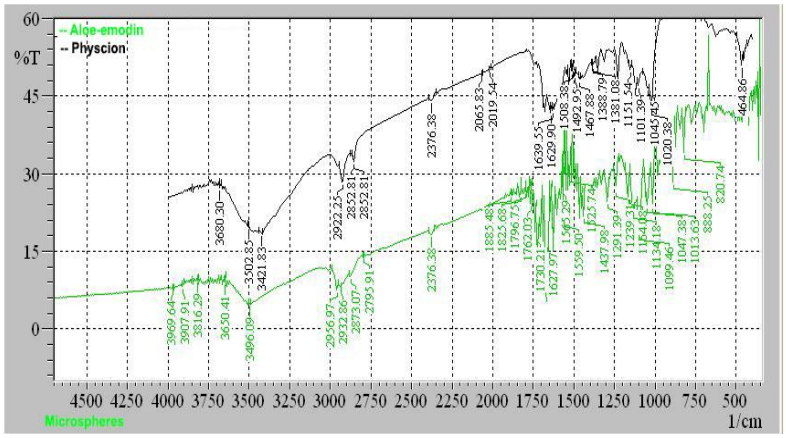

Figure 3. IR of aloe-emodin and physcion containing microspheres

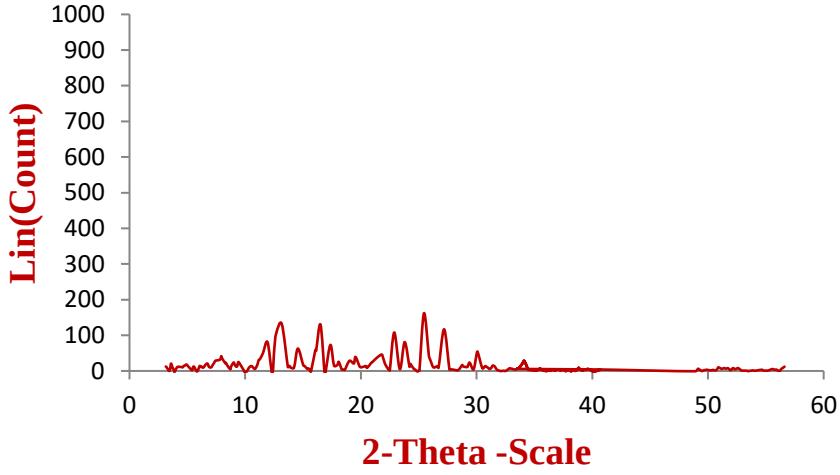

Figure 4. XRD of H.V. Aloe-emodin

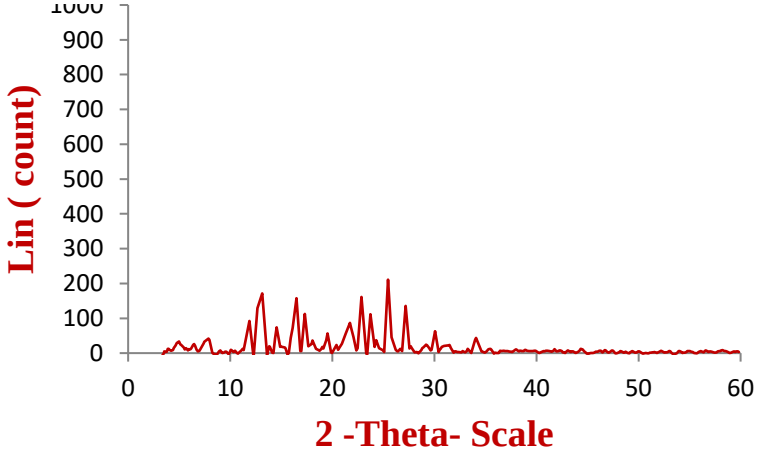

Figure 5. XRD of H.V. physcion

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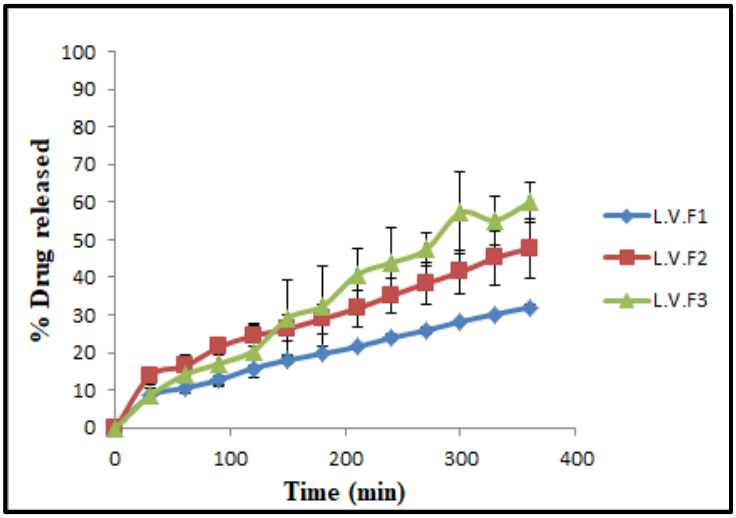

Figure 6. In vitro drug release microspheres of (L.V. batch)

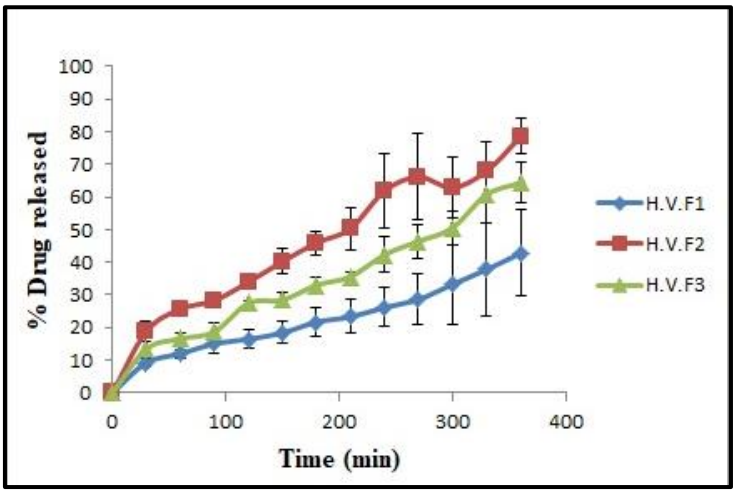

Figure 7. In vitro drug release of EPS microspheres (H.V. batch)

Antimicrobial activity

The combined Physcion emodin and processed aloe Microspheres were tested for their sensitivity to a range of concentrations and their MIC and MBC against Gram-positive and Gram-negative bacteria and Candida albicans, respectively, in vitro. The disc diffusion method was used to conduct the sensitivity test, and ciprofloxacin and fluconazole were used as the gold standards for antibacterial and antifungal activities, respectively, to determine the susceptibility of the microorganisms to the test microspheres. Zone of Inhibition (ZOI) diameter, which includes disc diameter, was used to evaluate sensitivity. *Bacillus subtilis* (MTCC 3165), *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 614), *Puedomonas aeruginosa* (MTCC 424), and *Candida albicans* (MTCC227) were all tested against the Microsphere. Table 8 shows the results of the antibacterial activity, while Figures 8, 9, 10, 11, and 13 show the corresponding images. The antimicrobial activity of aloe-emodin and physcion-containing microsphere at a concentration of 50 mg/ml against *Bacillus subtilis*, maximum ZOI was observed with aloe-emodin containing Microsphere (Maximum ZOI 19.20mm) and minimum with physcion containing Microsphere (Minimum ZOI 18.26 mm). Against *Staphylococcus aureus*, maximum ZOI was observed with aloe-emodin containing Microsphere (Maximum ZOI 20.21 mm) and minimum with physcion containing Microsphere (Minimum ZOI 18.20 mm), Against *Escherichia coli*, the maximum ZOI was observed with aloe-emodin containing Microsphere (Maximum ZOI 21.20 mm) and minimum with physcion containing Microsphere (Minimum ZOI 18.26 mm), Against *Pseudomonas aeruginosa*, maximum ZOI was observed with aloe-emodin containing Microsphere (Maximum ZOI 20.20 mm) and minimum with physcion containing Microsphere (Minimum ZOI 18.25 mm) and Against *Candida albicans*, the maximum ZOI was observed with aloe-emodin containing Microsphere (Maximum ZOI 19.15 mm) and minimum with physcion containing Microsphere (Minimum ZOI 16.12 mm) (Newman and Cragg, 2012; Frent et al., 2023).

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Table 8. Zone of inhibition against tested microorganisms

Concentration mg/ml	Aloe emodin Microsphere	Physician Microsphere	Ciprofloxacin (10µg/ml)
(*Bacillus subtilis)* Zone of Inhibition (mm)			
6.25	9.56	9.10	24.19
12.5	10.19	10.10	
25	12.36	12.45	
50	19.20	18.26	
(*Staphylococcus aureus)* Zone of Inhibition (mm)			
6.25	11.25	10.36	26.32
12.5	13.05	11.93	
25	16.19	14.26	
50	20.21	18.20	
(*Escherichia coli)* Zone of Inhibition (mm)			
6.25	10.5	10.2	25.41
12.5	13.11	12.22	
25	16.5	14.20	
50	21.20	18.26	
(*Pseudomonas aeruginosa)* Zone of Inhibition (mm)			
6.25	11.96	10.25	23.18
12.5	13.5	11.9	
25	17.20	15.33	
50	20.20	18.25	
(*Candida albicans)* Zone of Inhibition (mm)			
6.25	10.11	9.5	20.36
12.5	12.2	10.8	
25	14.18	12.11	
50	19.15	16.12	

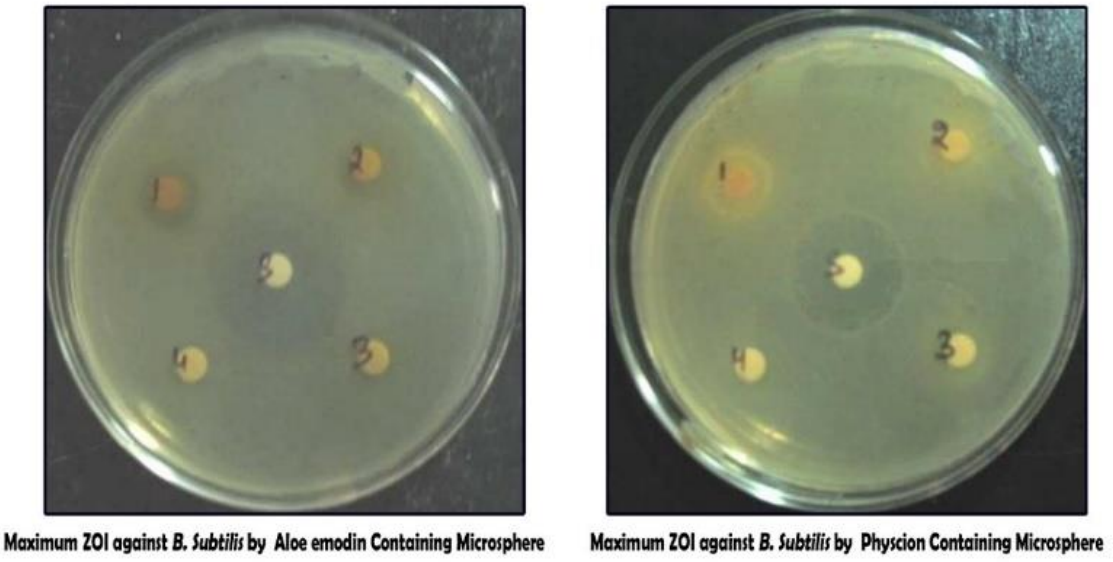

Figure 8. Maximum ZOI against *Bacillus subtilis* by aloe-emodin and physcion-containing microsphere

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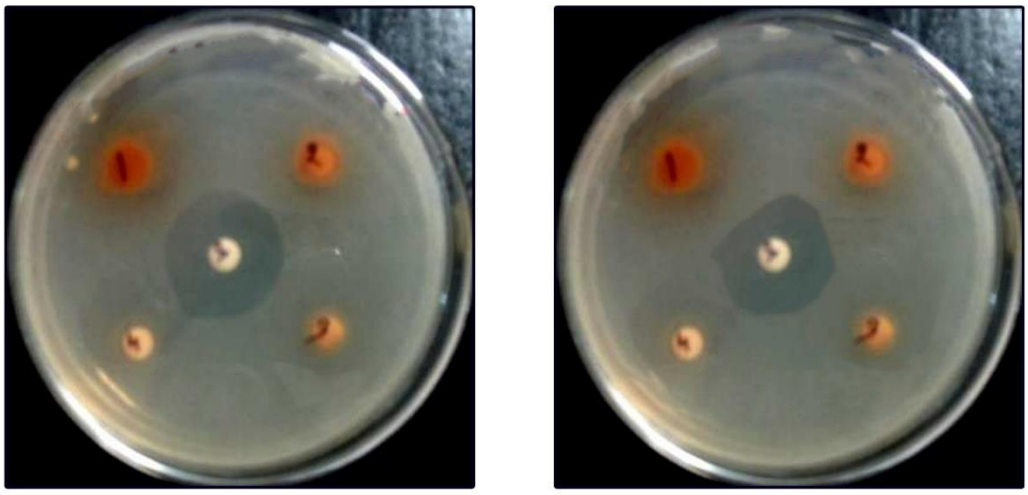

Figure 9. Maximum ZOI against *Staphylococcus* aureus by aloe-emodin and physcion containing microsphere

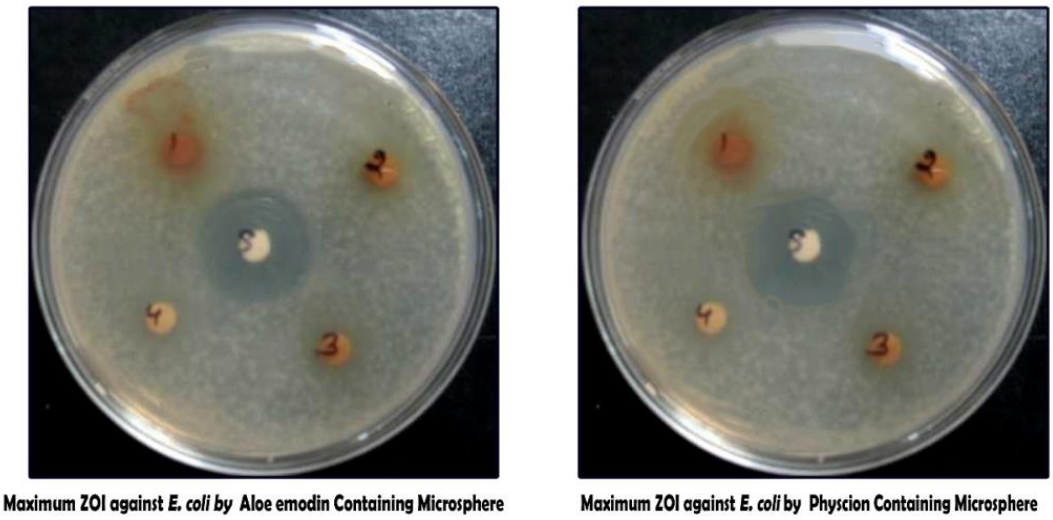

Figure 10. Maximum ZOI against *Escherichia coli* by aloe-emodin and physcion containing microsphere

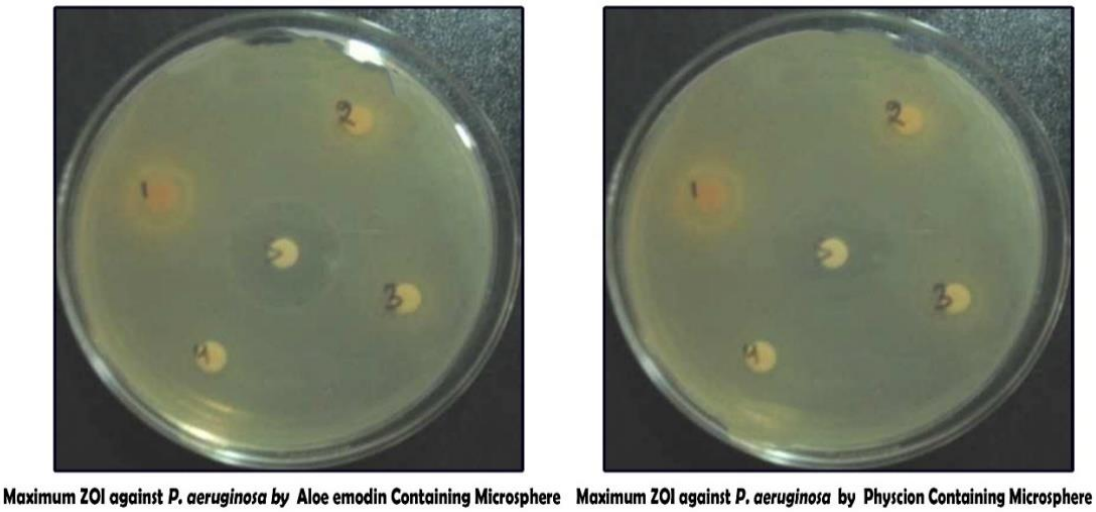

Figure 11. Maximum ZOI against *Puedomonasaeruginosa* by aloe-emodin and physcion containing microsphere

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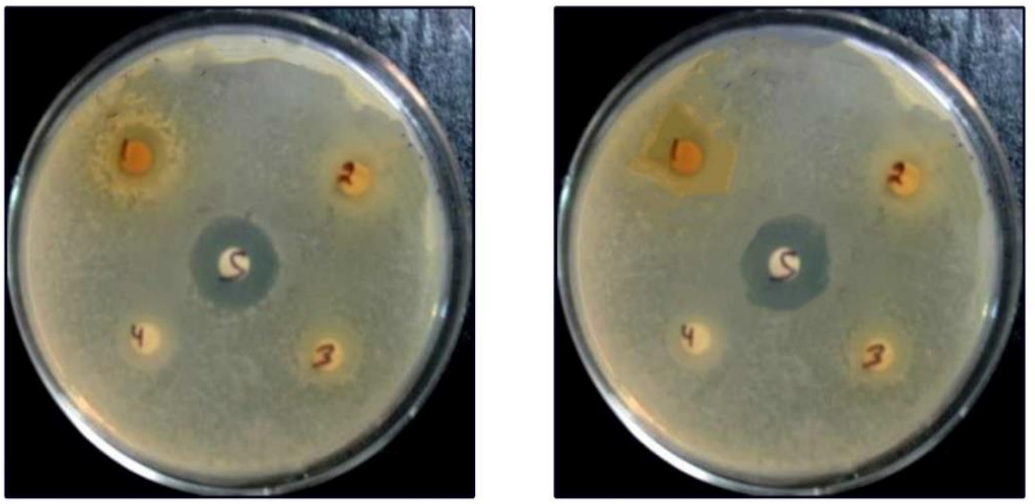

Figure 12. Maximum ZOI against *Candida albicans* by aloe-emodin and physcion containing microsphere

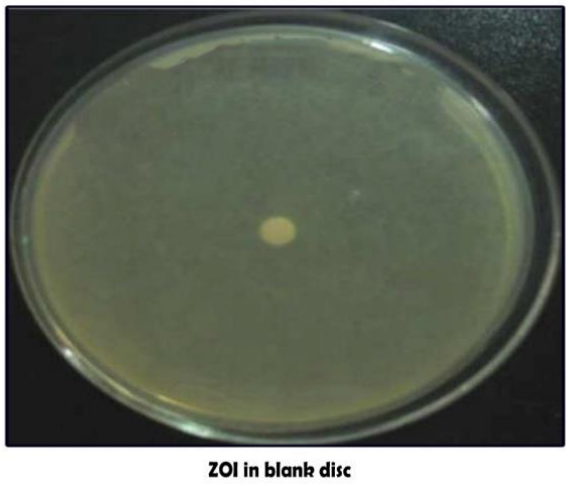

Figure 13. ZOI in blank disc

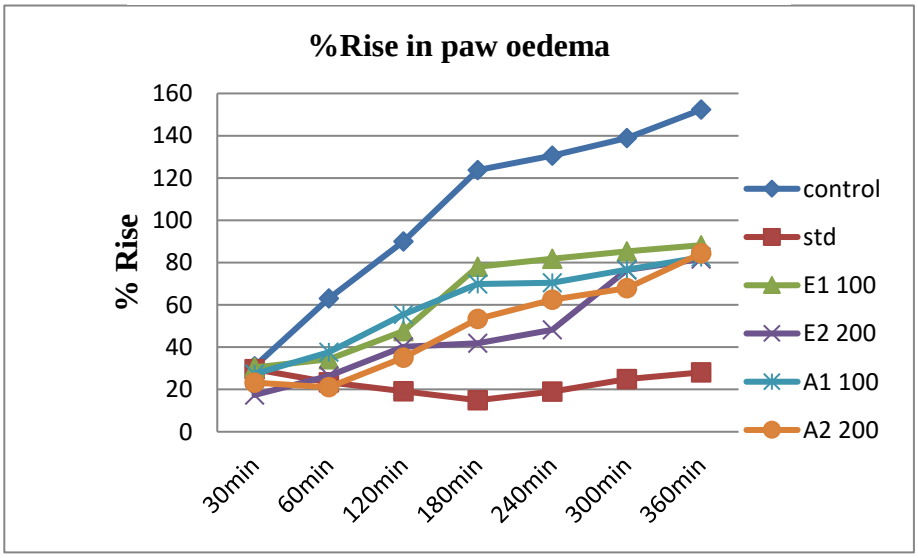

Figure 14. Anti-inflammatory activity indicates % rise in paw volume

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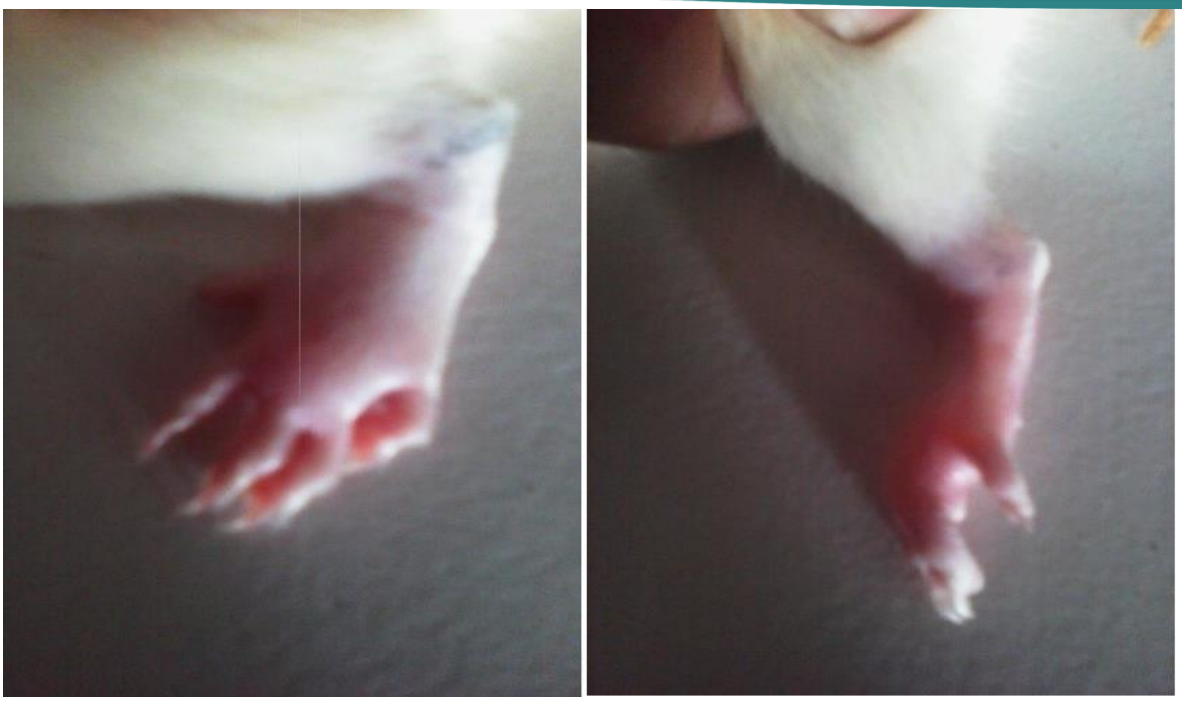

Figure 15. Inflamed, edematous hind paw after carrageenan injection

Pharmacological studies

Acute Oral Toxicity Study

Acute toxicity study of aloe-emodin and physician-containing microsphere did not produce any sign of toxicity, and no mortality occurred within 24h after administration and after 14 days observation at a single oral dose of 2000mg/kg body weight. Thus, 100 and 200 mg/kg doses were selected for pharmacological study (Hajare et al., 2023).

Anti-inflammatory activity

Producing pro-inflammatory chemicals (TNF-α, IL-1, IL-6) and activating oxidative stress leads to further tissue damage in the complex process known as inflammation. To discover chemicals with anti-inflammatory activity, carrageenan has been utilised extensively as a toxicant to cause experimental inflammation. Table 9 and Figure 14-15 tabulated the anti-inflammatory study results. The control group treated with carrageenan showed a study rise in paw volume, indicating inflammation was produced successfully. The standard drug diclofenac showed its effect from 60 min and remained consistent to till 6 h. The lower dose of Aloe emodin and physician (100 mg/kg) extract showed moderate inhibition, revealed by minimum % rise at the late phase (78.10 and 69.84 at 180^{th} min, $P<0.01$). While, at higher doses aloe-emodin and physician extracts at higher doses (200mg/kg) showed comparatively better anti-inflammatory activity, which was achieved at 30 min of carrageenan injection and continued up to the 240 min. The aloe-emodin at both dose levels was effective in the early phase of inflammation. According to the findings of this research, the chosen plant extracts have an immediate anti-inflammatory effect. There are three phases to the mediator release during carrageenan-induced inflammation: the first (from 0 to 2 hours), the second (3 hours), and the third (from >4 hours). Paw edoema caused by carrageenan was greatly reduced by the lower dose of aloe-emodin and Physcion (100 mg/kg) extract, suggesting an inhibitory impact on the release of histamine and/or serotonin. Whereas, at higher doses, aloe emodin and physcion (200 mg/kg) extract showed significant activity in the first two phases is indication that the activity was due to inhibition of serotonin, histamine and kinins (Park et al., 2023; Tare and Thube, 2009; Rajpoot et al., 2023).

4. Conclusion

This study was designed for the first time for the isolated alcoholic fraction of aloe-emodin and physcion by root extract containing microspheres to screen Cassia fistula's antimicrobial and pharmacological activities. Microparticulate delivery as plants are becoming probable source for phytoconstituents with wide-ranging pharmacological activities. Identification and characterization of such plants with potential phytoconstituents that can be used in herbal medicines is significant. As an overture to this, it becomes essential to study the plant's various pharmacognostical characteristics before further investigation. SEM is a valuable tool in characterizing microspheres and understanding their surface morphology. The information obtained from SEM images aids in optimizing the

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Table 9. Percentage increase in paw oedema (%inhibition) after Carrageenan administration

Group	Dose	%Increase in paw oedema (%inhibition)						
		30 min	60 min	120min	180min	240min	300min	360min
Control	10 ml /kg	19.23±5.67	63.034±8.73	90.01±7.35	123.80±8.51	130.54±11.76	138.84±14.97	152.33±15.33
Standard (diclofenac)	10 mg/kg	29.56±0.63*-	23.40±5.52***(11.26)	19.07±3.38***(61.28)	14.91±3.47***(100.64)	19.05±5.88***(103.62)	24.97±6.24***(107.79)	28.03±6.38***(119.97)
Physcion	100 mg/kg	27.33±1.57-	37.59±0.75***-	55.95±5.30***-	69.84±2.90***(35.42)	70.50±4.18***(44.73)	76.64±5.36***(49.45)	82.55±6.21***(64.03)
Physcion	200 mg/kg	23.60±1.40-	21.04±1.70***(6.99)	35.03±0.90***(26.58)	53.40±3.54***(52.32)	62.46±1.51***(51.02)	67.85±0.29***(56.17)	84.36±8.56***(59.39)
Aloe emodin	100 mg/kg	30.43±1.10*-	34.36±2.03***-	47.58±1.54***(18.07)	78.10±3.63***(37.13)	81.86±2.46***(42.76)	85.24±1.82***(50.81)	88.25±1.72***(68.60)
Aloe emodin	200 mg/kg	17.37±1.34-	26.45±1.35***(9.05)	40.14±3.21***(35.18)	41.83±1.33***(78.25)	48.21±4.19***(80.55)	76.50±6.96***(66.61)	81.70±8.28***(81.71

synthesis process, assess the microspheres' quality, and tailor their properties for specific applications. Scanning Electron Microscopical image of formulation was observed to be oval to spherical in shape. XRD studied the crystalline nature of microspheres with low and high-viscosity grade formulation. H.V. aloe emodin and Physcion loaded microspheres showed high intensity peak at 25.45º and 22.87º. It proved spray drying because encapsulation of sample in polymer matrix and calculation of the drug release kinetic from aloe emodin loaded microspheres release constant was calculated from slope of appropriate model and regression coefficient (R^2). Optimized batch H.V. aloe emodin was best fitted to the Higuchi model shown (R^2=0.904). The Korsmeyer-Peppas equation indicated an excellent linearity of regression (R^2=0.984). The release constant 'n' was found to be (0.730), and K value was 0.068. for the observations of antimicrobial activity, so it was concluded that the maximum ZOI was observed with aloe-emodin containing Microsphere against *Escherichia coli* (21.20 mm) and minimum ZOI with Physcion containing Microsphere against *Candida albicans*, (16.12 mm) at 50mg/ml. Even at lower concentrations, microspheres showed antimicrobial activity. MIC and Minimum MMC of aloe-emodin and Physcion containing microsphere against tested microorganisms. The anti-inflammatory activity of aloe-emodin and Physcion was statistically significant. Both microsphere samples were found capable of controlling the inflammation till 6 h. According to the findings of this research, the chosen plant extracts have an immediate anti-inflammatory effect. There are three phases to the mediator release during carrageenan-induced inflammation: the first (from 0 to 2 hours), the second (3 hours), and the third (from >4 hours). Microspheres containing aloe-emodin and Physcion demonstrate increasing percent inhibition from 120 minutes to 360 minutes, with maximum percent inhibition occurring at 360 minutes. Standard drugs show % inhibition of 11.26, 61.28, 100.64, 103.62, 107.79 and 119.97 on 60 min,120 min, 180 min,240 min,300 min and 360 min, respectively. Aloe emodin-containing microspheres showed maximum % inhibition of 76.50 % and 85.71 % at 100 and 200 mg /kg oral doses on 360 min, respectively.

References

Dama, G.Y., Tare, H. L., Gore, M. S., Shende, V. S., Deore, S. R., Khandagale, S. T., &Kandekar, A. E. (2011). Comparative cardiotonic activity of *Helicteresisora* with digoxin on isolated frog heart. *International Journal of Preclinical and Pharmaceutical Research*, *2*(2), 81-86.

Dhadde, G. S., Mali, H. S., Raut, I. D., Nitalikar, M. M., & Bhutkar, M. A. (2021). A review on microspheres: Types, method of preparation, characterization and application. *Asian Journal of Pharmacy and Technology*, *11*(2), 149-155. http://dx.doi.org/10.52711/2231-5713.2021.00025

Frent, O. D., Duda-Seiman, D. M., Vicas, L. G., Duteanu, N., Nemes, N. S., Pascu, B., ... & Marian, E. (2023). Study of the Influence of the Excipients Used for the Synthesis of Microspheres Loaded with Quercetin: Their Characterization and Antimicrobial Activity. *Coatings*, *13*(8), 1376. https://doi.org/10.3390/coatings13081376

Hajare, P., Rai, V., Nipate, S., Balap, A., Pimple, B., Chumbhale, D., ... & Tare, H. (2023). Anti-

arthritic potential of ethyl acetate fraction of Pinus roxburghii Sargent stem bark in Freund's complete adjuvant induced arthritis in Wistar rats. *Multidisciplinary Science Journal*, *5*(4), 2023046-2023046. https://doi.org/10.31893/multiscience.2023046

He, S., & Mu, H. (2023). Microenvironmental pH Modification in Buccal/Sublingual Dosage Forms for Systemic Drug Delivery. *Pharmaceutics*, *15*(2), 637. https://doi.org/10.3390/pharmaceutics15020637

Ju, Y., Zeng, H., Ye, X., Dai, M., Fang, B., & Liu, L. (2023). Zn2+ incorporated composite polysaccharide microspheres for sustained growth factor release and wound healing. *Materials Today Bio*, 100739. https://doi.org/10.1016/j.mtbio.2023.100739

Mane, V., Killedar, S., More, H., Salunkhe, S., & Tare, H. (2023). Development and Validation of a Novel Bioanalytical Method for Estimating Epigallocatechin 3 Gallate in Wistar Rat Plasma by RP-HPLC Employing Gradient Elution Techniques. *Journal of Research in Pharmacy*, *27*(3). https://dx.doi.org/10.29228/jrp.397

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of natural products*, *75*(3), 311-335. https://doi.org/10.1021/np200906s

Park, J. E., Kim, Y. K., Kim, S. Y., Choi, J. B., Bae, T. S., Jang, Y. S., & Lee, M. H. (2023). Biocompatibility and Antibacterial Effect of Ginger Fraction Loaded PLGA Microspheres Fabricated by Coaxial Electrospray. *Materials*, *16*(5), 1885. https://doi.org/10.3390/ma16051885

Rajpoot, S. R., Ahmad, K., Asif, H. M., Madni, M. A., Tasleem, M. W., Zafar, F., ... & Hayee, A. (2023). Study of anti-inflammatory and anti-arthritic potential of curcumin-loaded Eudragit L100 and hydroxy propyl methyl cellulose (HPMC) microparticles. *Polymer Bulletin*, 1-16. https://doi.org/10.1007/s00289-023-04899-y

Shreya, S., Jain, S. K., Guru, S. K., & Sahu, A. N. (2023). Anti-cancer Potential of Pleurotus Mushroom: Detailed Insight on the Potential Bioactive Molecules, In vitro-In vivo Studies, and Formulation. *Letters in Drug Design & Discovery*, *20*(4), 439-456. https://doi.org/10.2174/1570180819666220518100010

Tare, H. L., &Thube, B. B. (2009). Comparative cardiotonic activity of C. angustifolia with digoxin on perfused frog heart. *IJPRD. 2009; 1 (1): 1*, 14.

Yang, C., Zhang, Z., Gan, L., Zhang, L., Yang, L., & Wu, P. (2023). Application of Biomedical Microspheres in Wound Healing. *International Journal of Molecular Sciences*, *24*(8), 7319. https://doi.org/10.3390/ijms24087319

Zahid, N., Ejaz, M., Khalid, N., Ikram, N., Anwar, F., & Farooq, Z. (2023). Effect of Cassia Fistula Bark Hexane Extract on Lipid Parameters in Male Diabetic Rats. *Esculapio Journal of SIMS*, *19*(01), 82-85. https://doi.org/10.51273/esc23.2519117

Zhu, Y., Chen, T., Feng, T., Zhang, J., Meng, Z., Zhang, N., ... & Zhou, Y. (2023). Fabrication and Biological Activities of All-in-One Composite Nanoemulsion Based on Blumea balsamifera Oil-Tea Tree Oil. *Molecules*, *28*(15), 5889. https://doi.org/10.3390/molecules28155889

Zlatić, N., & Stanković, M. (2020). Anticholinesterase, Antidiabetic and anti-inflammatory activity of secondary metabolites of Teucrium species. *Teucrium Species: Biology and Applications*, pp. 391-411. https://doi.org/10.1007/978-3-030-52159-2_1

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