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Formulation and Evaluation of Microsphere of Oxalis Stricta Extracts and Its Anti-Microbial Activity

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ABSTRACT

Objective: The purpose of the present investigation was to Formulate and evaluate microsphere of Oxalis stricta extract and its anti-microbial activity
Methods: The extract-loaded microspheres using biological macromolecule ethyl cellulose (EC) was prepared by solvent evaporation method using HPMC polymer. The effects of different process and formulation variables (stirring speed, evaporation time, and drug/polymer ratio) on microsphere properties were investigated.
Results: SEM revealed the spherical nature of the produced microspheres. Particle size study revealed that the average particle size of microspheres ranges from 798.9 to 959.3 nm. These particle size values indicate that the all formulated microsphere is under the range of microsphere and F2 is the lowest particle size of all formulation and Zeta potential was found to be all formulation range -1.7 to -4.1 mV with peak area of100% intensity. The formulation remained physically and chemically stable for three months under accelerated stability conditions (250C±2 0C and 60 ± 5% RH) and (400C±2 0C and 70 ± 5% RH).
Conclusion: The current study's findings indicate that microsphere formulations are a promising carrier for new herbal medication delivery.

Keywords: Microspheres, Ethyl cellulose, Oxalis stricta, Solvent evaporation, Novel drug delivery system.

1. INTRODUCTION

Medicinal plants have long been a cornerstone of traditional medicine systems worldwide, offering a vast array of bioactive compounds with therapeutic potential. Among such plants, *Oxalis stricta* (commonly known as yellow wood sorrel) has garnered interest due to its diverse pharmacological properties. Native to North America and parts of Asia, *O. stricta* is traditionally used for its diuretic, anti-inflammatory, and antimicrobial effects **(Ahmed and Beg, 2001; Nadkarni and Nadkarni, 1967; Kirtikar and Basu, 1918)**. The emergence of multidrug-resistant microbial strains has necessitated the exploration of new antimicrobial agents, especially those derived from natural sources. Phytochemicals such as flavonoids, tannins, alkaloids, and phenolic compounds present in *O. stricta* have demonstrated promising antimicrobial properties in previous studies **(Cowan, 1999; Parekh and Chanda, 2007; Doughari, 2012)**. However, the direct application of plant extracts often faces limitations such as poor stability, low bioavailability, and short half-life, which can hinder their therapeutic potential **(Meihua, 2015)**.To overcome these challenges, the incorporation of plant extracts into Novel medication delivery techniques, such as microspheres, has been extensively investigated. Microspheres, being biodegradable and biocompatible carriers, offer several advantages including controlled drug release, protection of encapsulated bioactives, and enhanced therapeutic efficacy **(Jain, 1997; Barbosa *et al.*, 2019; Mundargi *et al.*, 2008)**. The formulation of microspheres using natural or synthetic polymers such as sodium alginate, chitosan, and PLGA (poly(lactic-co-glycolic acid)) it has been proven to improve the pharmacokinetics of various phytoconstituents **(Patil and Sawant, 2011; Soppimath *et al.*, 2001; Das and Senapati, 2008)**.Encapsulation of *O. stricta* extracts into microspheres could significantly enhance their antimicrobial action by allowing sustained release and better penetration to the site of infection **(Salim, 2020; Oyenihi *et al.*, 2021)**. Furthermore, this strategy could minimize the dosage frequency and reduce potential side effects, making it a viable alternative in antimicrobial therapy **(Dhanaraj *et al.*, 2016; Patel and Amin, 2011; Agnihotri *et al.*, 2004)**.In few recent years, the pharmaceutical industry has aimed on developing plant-based microsphere formulations to address resistance to antibiotics and improve the therapeutic index of herbal medicines. Studies have demonstrated that microsphere-based delivery systems can preserve the integrity of volatile and sensitive phytochemicals, ensuring prolonged activity **(Pandey and Khuller, 2005; Saadh *et al.*, 2024)**. Therefore, this study aims to formulate and evaluate microspheres containing *Oxalis stricta* extracts and investigate their antimicrobial activity

against selected bacterial and fungal strains. This research not only aids in the advancement of plant-derived therapeutics but also supports the global initiative towards sustainable and natural alternatives to conventional antibiotics. A detailed investigation into the physicochemical properties, encapsulation efficiency, profile of release, and antimicrobial effectiveness of the prepared microspheres will provide critical insights into their potential application in pharmaceutical and clinical settings.

2. MATERIAL AND METHOD

2.1 Plant collection

The medicinal plant *Oxalis stricta* (300 gm) was collected. After cleaning, plant parts (leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

2.2 Extraction

The Soxhlet apparatus and a continuous hot percolation method were used in the current experiment to extract plant material. A soxhlet apparatus thimble was filled with powdered *oxalis stricta*. Soxhlation was performed at 60°C using petroleum ether as a non-polar solvent. The depleted vegetation (marc) was extracted again using a methanol solvent after it had dried. The extraction procedure was repeated for each solvent until there was no longer any visible color shift in the siphon tube. The extraction was considered successful when there was no more solvent remaining after it evaporated. A Buchi-style rotating vacuum evaporator was used to evaporate the extracted materials at 40°C. The prepared extract was labeled for future use and stored in an airtight container after being examined for organoleptic characteristics (percentage yield, color, and odor) **(Baidya *et al.*, 2002).**

2.3 Phytochemical investigation

An experiment was conducted to ascertain whether certain phytoconstituents were present or absent using a thorough qualitative phytochemical examination. The colour intensity or the precipitate formation was used as medical responses to tests **(Priyank *et al.*, 2011)**.

2.4 Solubility study

Qualitative solubility of *Oxalis stricta* in different solvents was determined according to USPNF, 2007. Approximately 1 mg of *Oxalis stricta* was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, DMOS ethanol, and water) **(Jain and Verma, 2020).**

2.5 Microsphere formulation using the solvent evaporation method

Using the solvent evaporation process, extract (Oxalis stricta) was utilized as the main ingredient to create microspheres. At room temperature, EC, HPMC, and extract (Oxalis stricta) were dissolved in a 1:1 ethanol and dichloromethane solution. This was added to 250 milliliters of water with 0.01% Tween-80 that was kept between 30 and 40 degrees Celsius. The mixture was then agitated for 45 minutes at 300 rpm to allow the volatile solvent to evaporate. After filtering and washing with water, the microspheres were dried in an oven set at 37°C **(Fartyal *et al.*, 2011)**.

Table 1: Formulation of the microsphere composition

Formulations (Code)	Polymer HPMC(mg)	Polymer Ethyl cellulose (mg)	Extract (mg)	Temperature °C	Solvent ratio(1:1) ethanol/DCM
F1	300	50	100	30-40°C	5ml:5ml
F2	250	100	100	30-40°C	5ml:5ml
F3	200	150	100	30-40°C	5ml:5ml
F4	150	200	100	30-40°C	5ml:5ml
F5	100	250	100	30-40°C	5ml:5ml

2.6 Parameter for evaluating the extract-loaded microsphere

2.6.1 Size of particles

Microspheres were diluted with Millipore filtered water to the proper scattering intensity at 25°C, and their sizes were measured using the Malvern Zeta sizer before being put in a disposable cuvette **(Singh and Vingkar, 2008).**

2.6.2 Zeta potential

Microspheres diluted ten times with distilled water and sonicated for five to fifteen minutes were evaluated using Zeta sizer Malvern equipment to determine the zeta potential, which is used to assess particle charge and movement velocity in an electric field **(Singh and Vingkar, 2008).**

2.6.3 Scanning Electron Microscopic (SEM)
The morphological properties of extract-loaded microspheres were determined using a scanning electron microscope. A vacuum-sputter coater coated the microspheres with gold, palladium, or platinum. The specimen was exposed to an electron beam, causing secondary electrons to develop. Only 90° dispersed electrons were chosen, and processed using Rutherford and Kramer's Law to acquire surface topography images **(Volic *et al.*, 2022).**

2.7 Anti-bacterial activity of Microsphere by Well diffusion assay
The process of creating Nutrient Agar Media involved dissolving 28g of Nutrient Media in 1 litre of distilled water, checking its pH before sterilization, and sterilizing it in an autoclave at 121ºC at 15lbs pressure for 15 minutes. The agar was then solidified in laminar air flow. The bacterial suspension of *E. coli* was standardized to 108 CFU/ml and transferred to a fresh and sterile solidified Agar Media Plate. The inoculums were spread over the entire surface, and three wells of 6mm were formed for the inoculation of the microsphere, Microsphere, and extract solution. 100 µl of the sample was loaded and allowed to diffuse for 30 minutes at room temperature. The plates were then incubated for 18-24 hours at 37ºC. A clear zone of inhibition (ZOI) was measured in millimeters, and the diameters of the zone of complete inhibition were measured, including the well diameter **(Ahmed *et al.*, 2020; Mohammadi *et al.*, 2012)**.

2.8 Studies of stability
For three months, the extract-loaded microspheres were kept stable at various humidity and temperature settings in a stability test chamber. At various intervals, the formulation was examined for zeta potential and particle size investigations. In accordance with ICH requirements, it was also tested for stability during three months of accelerated storage conditions. The final formulation of 0 days served as the baseline for comparing the results.

3. RESULTS

3.1 Percentage Yield
When extracting phytochemicals, the yield % is very crucial in order to define the standard efficiency of extraction for a specific plant, various sections of the various solvents or the same plant. The extracts' yield received from the *Oxalis stricta* is shown in Table: 2

Table 2: Percentage Yield of crude extracts of *Oxalis stricta* extract

S.No	Plant name	Solvent	Theoretical weight	Yield(gm)	%yield
1	*Oxalis stricta*	Pet-ether	299.23	1.49	0.49%
2		Methanol	289	6.56	2.26%

3.2 Preliminary Phytochemical study

Table3: Phytochemical testing of extract

S. No.	Experiment	The existence or lack of a phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Present	Absent
1.2	Mayer's reagent test	Present	Absent
1.3	Wagner's reagent test	Present	Absent
1.3	Hager's reagent test	Present	Absent
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling'stest	Absent	Present
3.3	Benedict'stest	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**ProteinsandAminoAcids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Absent
5.2	Lead Acetate test	Absent	Absent
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Absent

7.	**Saponin**		
7.1	Foam test	Present	Present
8	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

3.3 Solubility study

Table 4: Solubility study of Extract

Extract	Solvents	Observation/Inference
Oxalis stricta	Methanol	Freely Soluble
	Ethanol	Soluble
	DMSO	Soluble
	Water	insoluble

The soluble nature of Oxalis stricta extract was tested in various liquid vehicles like Dimethyl sulfoxide, methanol, ethanol, and water, with the results showing that the extract is easily dissolved in methanol and ethanol and DMSO.

3.4 Evaluation metric for compositions of microspheres

3.4.1 Determination of particle size

Figure 1: Particle size (F1), (F2), (F3), (F4) and (F5)

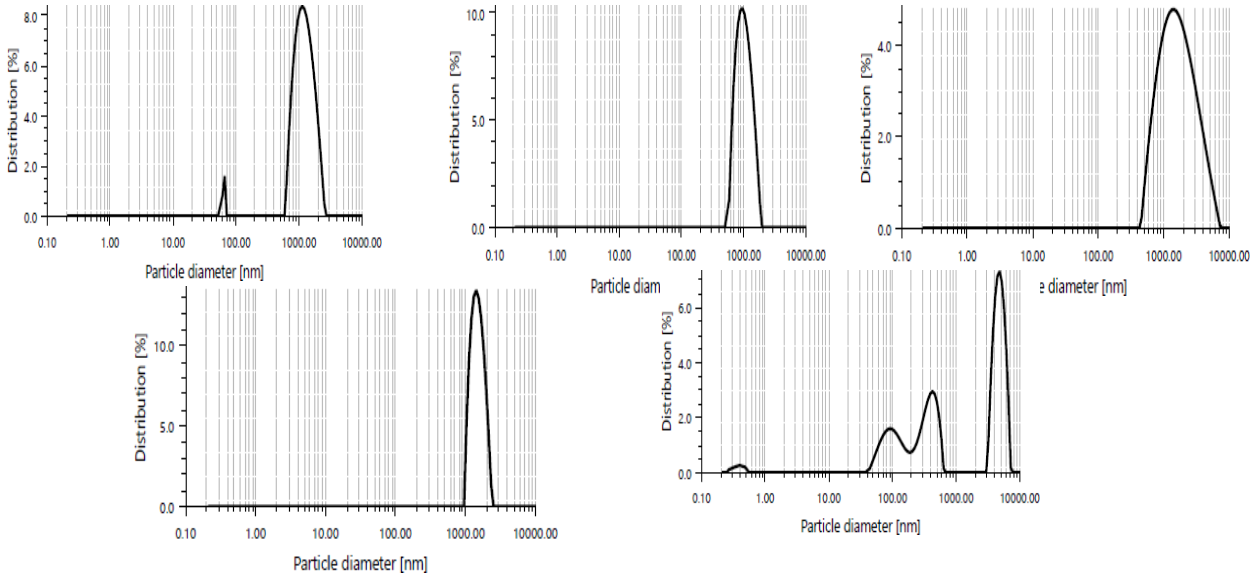

Table 5: The outcome of each formulation's particle size

S.No.	Formulations	Particle size (nm)	PI Value
1.	F1	919.7nm	1.045
2.	**F2**	**798.9nm**	**0.123**
3.	F3	859.6nm	1.760
4.	F4	898.9nm	0.375
5.	F5	959.3nm	0.389

The study measured the typical particle sizes of a microsphere formulation using the Malvern zeta sizer, revealing an average range of 798.9 to 959.3 nm. These results indicate that all formulated microspheres fall within the microsphere range, with F2 being the lowest particle size in all formulations, as shown in Table 5.

3.4.2 Zeta potential determination

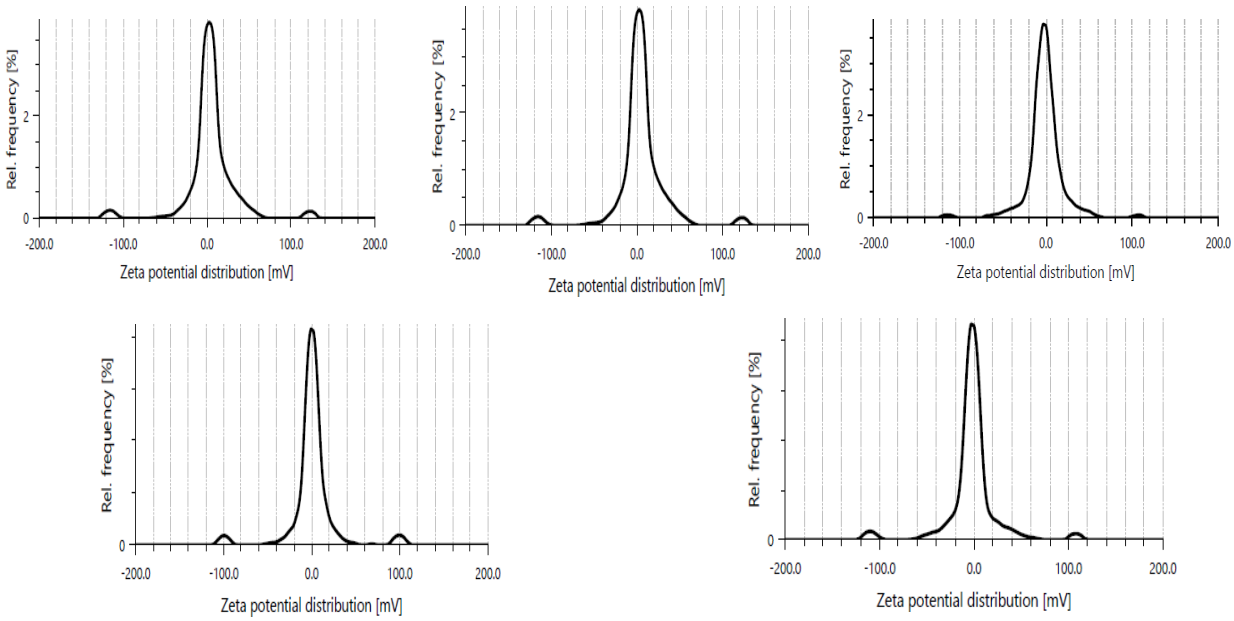

Figure 2: Zeta potential (F1), (F2), (F3), (F4) and (F5)

Table 6: Result of Zeta potential of all formulations

S.No	Formulation	Zeta potential
1	Microsphere(F1)	-1.7mV
2	**Microsphere(F2)**	**-1.9mV**
3	Microsphere(F3)	-4.1mV
4	Microsphere(F4)	-2.5mV
5	Microsphere(F5)	-2.2mV

Analysis of zeta potential is utilized to determine the charge on the surface of particles, with a magnitude predicting colloidal stability. The zeta potential ranges from -1.7 to -4.1 mV, with a 100% intensity peak, indicating the consistency of the all-formulated microsphere.

3.4.3 Scanning electron microscopy characterization of F2 formulation

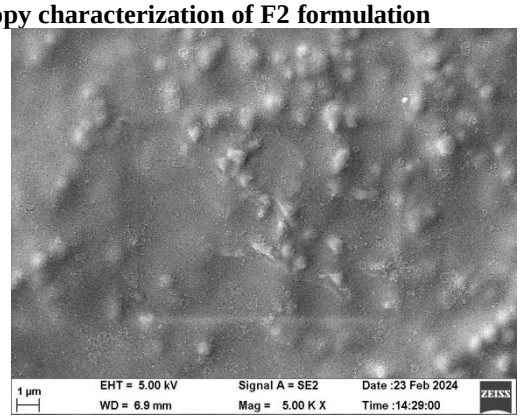

Figure 3: SEM

Scanning electron microscopy (SEM) was used to examine the microsphere and ascertain its microscopic properties. The microspheres were found to have a spherical form and a smooth surface morphology following preparation and drying. These features were revealed by the SEM scans, as seen in Figure 3.

3.5 Results of antimicrobial activity of microsphere F2 formulation

3.5.1 The antimicrobial properties of Formulation against *E.coli*

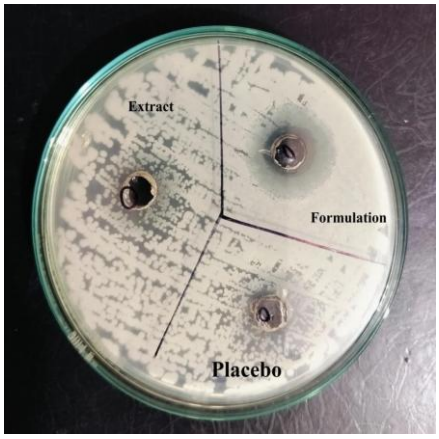

Figure 4: Anti-microbial activity

Table7: Anti-microbial activity of Formulation, placebo and plant extract against *E.coli*

S.No.	Sample Name	Zone of Inhibition(mm)
1.	Placebo	6mm
2.	Plant extract	10mm
3.	Formulation	15mm

3.6 Stability study

Table8: Analysis of Microsphere Stability (F2) formulation

S. No	Time (Days)	$25^0C±2^0C$and60±5%RH			$40^0C±2\ ^0C$ and70±5%RH		
		Appear ance	Particle sizenm	Zeta potential mV	Appearance	Particle size nm	Zeta potential mV
1.	0	Solid Powder	798.9nm	-1.9mV	Solid Powder	798.9nm	-1.9mV
2.	30	Solid Powder	799.2nm	-2.1mV	Solid Powder	799.7nm	-2.3mV
3.	45	Solid Powder	799.8nm	-2.5mV	Solid Powder	800.2nm	-2.5mV
3.	60	Solid Powder	800.1nm	-2.9mV	Solid Powder	801.1nm	-2.8mV
4.	90	Solid Powder	800.9nm	-3.1mV	Solid Powder	801.8nm	-3.5mV

The formulation remained stable for three months under conditions of rapid stability (250C±2 0C and 60 ± 5% RH) and (400C±2 0C and 70 ±5% RH), with no significant changes in physicochemical parameters like appearance, Zeta potential, and particle size.

4. DISCUSSION

The current investigation concentrated on the development and assessment of microspheres incorporating *Oxalis stricta* extracts, aimed at enhancing their antimicrobial efficacy and overcoming challenges associated with the direct use of plant-based therapeutics. The choice of ethyl cellulose and HPMC as polymers for microsphere fabrication via technique of solvent evaporation was appropriate, considering their established roles in achieving stability and regulated medication release in drug delivery systems. The prepared microspheres' particle size analysis revealed a range between 798.9 nm to 959.3 nm, confirming their classification within the microscale. Among all formulations, F2 demonstrated the smallest particle size (798.9 nm) with a polydispersity index (PDI) of 0.123, indicating a uniform particle distribution and better stability. Zeta potential values for all formulations ranged from -1.7 to -4.1 mV, which, although slightly low in absolute terms, suggest moderate stability. The F2 formulation again showed favorable stability parameters throughout the study. SEM examination of the F2 formulation verified the microspheres were spherical with smooth surface morphology, which is essential for consistent drug release and bioavailability. This structural integrity suggests successful encapsulation and good formulation characteristics. The antimicrobial assessment using the well diffusion assay revealed significant enhancement in bioactivity post encapsulation. While the crude *Oxalis stricta* extract showed a zone of inhibition (ZOI) of 10 mm against *E. coli*, the microsphere

formulation (F2) exhibited a larger ZOI of 15 mm, clearly indicating improved antimicrobial action due to sustained release and better penetration. The placebo showed negligible activity (6 mm), establishing the efficacy was derived from the active extract. Phytochemical screening confirmed presence of substances that are bioactive like alkaloids, glycosides, and saponins, predominantly in the methanolic extract. These constituents are likely in charge of the observed antimicrobial effect, aligning with previous findings on *Oxalis stricta*. Stability studies conducted over a time frame of 90 days under accelerated conditions showed minimal changes in appearance, particle size, and zeta potential, affirming the physical and chemical stability of the microsphere formulation. These findings further endorse the feasibility of microsphere-based distribution of herbal actives for long-term storage and practical application. Collectively, the results affirm that microsphere encapsulation significantly improves the therapeutic profile of *Oxalis stricta* extracts, providing a more efficient, stable, and targeted approach to antimicrobial treatment.

5. CONCLUSION

In conclusion, the formulation and assessment of microspheres containing *Oxalis stricta* extracts demonstrated promising potential as a innovative medication delivery method with significant antimicrobial activity. The microspheres were successfully developed using suitable polymers, exhibiting desirable physicochemical properties such as regulated release and stability. Antimicrobial testing revealed effective inhibition against a range of pathogenic microorganisms, supporting the traditional use of *Oxalis stricta* in herbal medicine. These findings suggest that *Oxalis stricta* microspheres might be a valuable candidate for future development of plant-based antimicrobial therapies.

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