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# Formulation and evaluation of biodegradable microsphere of plant extract of "pongamia pinnata"

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## ABSTRACT

The present study focuses on the formulation and evaluation of biodegradable microspheres containing plant extract of *Pongamia pinnata* (Karanja) for their antimicrobial activity. Pongamia pinnata, a plant renowned for its medicinal properties, was chosen for its bioactive compounds with potential therapeutic benefits. The primary objective was to develop a delivery system that enhances the stability and efficacy of the plant extract while ensuring controlled release and biodegradability. Microspheres were formulated using a solvent evaporation technique, employing biodegradable polymers such as HPMC and EC. The *Pongamia pinnata* extract was meticulously incorporated into the polymer matrix to form microspheres. Various parameters such as particle size, encapsulation efficiency, and surface morphology were characterized using scanning electron microscopy (SEM). The antimicrobial activity of the formulated microspheres was evaluated against common pathogenic microorganisms including *Escherichia coli* using the well diffusion method. The results demonstrated significant antimicrobial activity, indicating that the encapsulated extract retained its bioactivity and was effectively released from the microspheres. The study concluded that the biodegradable microspheres of *Pongamia pinnata* extract not only provide a sustained release of the bioactive compounds but also enhance the antimicrobial efficacy. This innovative approach holds promise for developing effective and eco-friendly antimicrobial agents, paving the way for further research in the domain of plant-based therapeutics.

**Keywords:** Biodegradable Microspheres, *Pongamia Pinnata*, Antimicrobial Activity, Solvent Evaporation Technique, Plant-Based Therapeutics.

## 1. INTRODUCTION

Drug delivery systems (DDSs) are pharmaceutical devices that facilitate targeted delivery of therapeutic agents in the body. They help transport active ingredients across biological barriers, contributing to the understanding of physiological barriers and the development of new drug delivery modes **(Nakmode *et al.*, 2023).** Microspheres are polymeric particles ranging from 1 to 1000 µm used for drug delivery. They can be classified into natural and synthetic types based

on their composition. Natural polymers include carbohydrates and proteins, while synthetic polymers include nonbiodegradable and biodegradable materials. Microspheres can be encapsulated or entrapped, making them a valuable tool in drug delivery **(Joshi *et al.*, 2023; Prasad *et al.*, 2014)**. Microspheres in drug delivery are used for targeted as well as prolonged drug release in the diseased area. It also protects the unstable or pH-sensitive drugs before and after the administration. Microspheres are classified into four different types: liposomes, Micelles, Microspheres and Microbubbles **(Abbasian *et al.*, 2024)**. Microspheres are utilized in various industries, including membrane technology, cell biology, and pre-transplant management of hepatocellular carcinoma. They are also used in carbonless copying paper, photosensitive paper, and fragrances like "scent-strips" and "scratch-n-sniff." Microcapsules are also used in diagnostic tests and biotech for producing recombinant and protein products **(Sahil *et al.*, 2011).** Chitosan and cellulose fibers, with high, intermediate, and low bile acid-binding capacities, prevented serum cholesterol levels in mice fed a high fat/high cholesterol diet from increasing by 2-fold and reduced cholesterol accumulation in hepatic stores **(Nair *et al* ;2009).** Chitosan polymer enhances drug stability by complexing with it, creating a dough mass, and passing it through a sieve to create stable granules. Chitosan, a biopolymer with osteoconductive, wound healing, and antimicrobial properties, is used as a bioactive coating for orthopedic and craniofacial implant devices, promoting tissue growth and bone regeneration **(Nair *et al* ;2009)**. Due to its therapeutic efficacy we have developed the microspheres containing bioactive molecules of plant extract. *Milletiapinnata*(L.) Pierre (Family: Leguminosae) is an important non-edible minor oilseed tree thatis a very fast-growing medium size plant with an average height of 30-40 feet and spreads canopy for casting moderate shade It can grow in various types of soil like alkaline, salty, sandy, clay, stony and waterlogged soils and also it shows high tolerance against drought bearing temperature up to 50°C. It is probably originated from India and grows naturally in India, Pakistan, Bangladesh, Malaysia, Vietnam, Thailand, Florida, Australia, and Sri Lanka and also in northeastern Australia, Japan, Fiji, and the Philippines **(Mukta *et al.*, 2010)**. It contains various phytoconstituents belonging to alkaloids, glycosides, flavonoids, fixed Oils, and carbohydrates category. Every part of this plant is used as crude drug for the treatment of various diseases due to the presence of Specific Chemical constituents **(Khatri and Patel 2013).** Milletiapinnata plant's flowers treat bleeding hemorrhoids, fruits treat abdominal ulcers, tumors, and hemorrhoids, seed powder reduces fever, leaves juices treat leprosy, diarrhea, coughs, gonorrhea, flatulence, and colds, bark reduces spleen swelling, root is used for oral hygiene, and oil stops bleeding and is beneficial for leprosy, piles, liver pain, and ulcers **(Warrier *et al.*, 1995).**

## 2. MATERIAL AND METHOD

### 2.1 Selection and collection of Plant: -

Plant and plant parts was selected on the basis of ethno-botanical survey. Pharmacological investigations report and recent investigations were considered in respect of selected Plant. Observe that selected plant should possess the following properties: -

- Anti- ulcer activity
- Anti- oxidant activity

- Anti- inflammatory activity, anti- microbial activity etc.
- Having good quality and quantity of bio-constituents.

Fresh leaves of *Pongamia pinnata,* free from disease were collected from local area. 340.12 gram of the powder prepared from shade-dried seed was subjected to extraction by soxhlation method, for 24 hours using solvent (ex- Petroleum ether, Methanol) as non-polar solvent at first.

**2.2 Extraction**

- **Soxhlet extraction method:**
- In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Pongamia pinnata* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethanol solvent. For each solvent, soxhlation was continued till no visual colour change will observed in siphon tube and completion of extraction were confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporate using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

- Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya *et al.*, 2002).**

**2.3 Determination of Percentage yield**

The percentage yield of *Pongamia pinnata* were determined as percentage of the weight of the extracts to the original weight of the dried sample used, using the formula **(Duniya *et al.*, 2018)**;

**Formula:**

$$\textbf{\% yield} = \frac{\textbf{Weight of extract}}{\textbf{Weight of plant material used}} \textbf{ x 100}$$

**2.4 Solubility Determination**

Solvent is a liquid that serves as the medium for reaction. It can serve two major purposes:

- Non-participatory to dissolve the reaction.
- Participatory a source of acid (protons), base (removing protons) or as a nucleophile (donating lone pair of electron).

Polarity is a separation of electric charge leading to a molecule or its chemical groups having an electric dipole moment, with a negatively charged end and positively charged end **(Jain and Verma 2020).**

**2.5 Phytochemical investigation: -**

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents. **(Yadav and Agarwala, 2011)**

## 2.6 Quantitative Tests

### A. Spectrophotometric Quantification of Total Phenolic Content: -

**Procedure–**Folin-Ciocalteu Assay was used for the determination of the total phenolic content in plant extract. The extracts (0.1 mL and 1mg/ml) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate and then the resulting solutions were allowed to stand for 30 minutes at room temperature before the absorbance was read spectrophotometrically. Subsequently, they were diluted to 5 mL and the absorbance was read instantly at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 mg/mL of Gallic aid was prepared. A blank solution was also prepared for the same situation and reagents for the preparation of the standard and sample solutions. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically **(Tangco *et al.*, 2015).**

### B. Spectrophotometric Quantification of Total Flavonoid Content: -

**Procedure**-Aluminium chloride colorimetric method **(Chang *et al.*, 2002)** was used for the determination of flavonoid content. 1 ml of each extract solution was mixed with 2.5ml of distilled water. Then, 75µl of sodium nitrite was added and mixed. After this stand for3 minutes before adding 0.15ml Aluminium chloride (100g/L) was added and allowed to stand for 5 minutes. Then, 0.5ml of 1 M sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. The calibration curve was calculated using Rutin as the standard. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy *et al.*, 2009).**

## 2.7 Formulation of microspheres by Solvent Evaporation method

Microspheres containing extract (*Pongamia pinnata*) as a core material were prepared by Solvent Evaporation method. Extract (*Pongamia pinnata*), HPMC and EC were dissolved in a mixture of ethanol and dichloromethane (1:1) at room temperature (As in table 1). This was poured into 250 mL water containing 0.01% Tween-80 maintained at a temperature of 30–40 °C and subsequently stirred at 300 rpm agitation speed for 45 minutes to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried in oven at 37°C. **(Fartyal *et al.*, 2011).**

**Table 1: Composition of microsphere formulation**

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

## 2.8 Evaluation parameter of extract loaded microsphere

### 2.8.1 Particle size

The particle size is one of the most important parameter for the characterization of microspheres. The size of microspheres was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette **(Singh and Vingkar 2008).**

### 2.8.2 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the microspheres was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements **(Đorđević *et al.*, 2015).**

### 2.8.3 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded microspheres were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography **(Anwer *et al.*, 2019).**

## 2.9 Anti-microbial activity

### 2.9.1 Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121$^{o}$C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

### 2.9.2 Well Diffusion Assay

The bacterial suspension of *E. Coli* was standardized to $10^8$ CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing $10^8$ CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate[33]**.** The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the noisome loaded Gel (0.5, 1 and 2 mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37$^{o}$ C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held

on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.

### 2.10 Stability studies

The Extract loaded Microsphere formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ($30^0C \pm 2^0C$ and 60 ± 5% RH) and ($40^0C \pm 2^0C$ and 70 ±5% RH) for 3 months. The formulation was checked for evaluation parameter particle size and Zeta potential studies at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter particle size and zeta potential studies.

All Results were compared against final formulation of 0 days as the reference.

## 3. RESULTS AND DISCUSSION

### 3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Pongamia pinnata*is shown in Table: 2

**Table 2: Percentage Yield of crude extracts of *Pongamia pinnata* extract**

| S.no | Plant name | Solvent | Theoretical weight | Yield(gm) | % yield |
|---|---|---|---|---|---|
| 1 | *Pongamia pinnata* | Pet ether | 300 | 1.37 | 0.46% |
| 2 | | Methanol | 284.25 | 6.59 | 2.32% |

### 3.2 Preliminary Phytochemical study

**Table 3: Phytochemical testing of extract**

| **Experiment** | **Presence or absence of phytochemical test** | |
|---|---|---|
| | **Pet. Ether extract** | **Methanolic extract** |
| Alkaloids | | |
| Dragendroff's test | Absent | Present |
| Mayer's reagent test | Absent | Present |
| Wagner's reagent test | Absent | Present |
| Hager's reagent test | Absent | Present |
| Glycoside | | |
| Borntrager test | Absent | Present |
| Legal's test | Absent | Present |
| Killer-Killiani test | Absent | Present |
| Carbohydrates | | |
| Molish's test | Absent | Absent |
| Fehling's test | Absent | Absent |
| Benedict's test | Absent | Absent |
| Barfoed's test | Absent | Absent |

| | | |
|---|---|---|
| Proteins and Amino Acids | | |
| Biuret test | Absent | Absent |
| Flavonoids | | |
| Alkaline reagent test | Absent | Present |
| Lead Acetate test | Absent | Present |
| Tannin and Phenolic Compounds | | |
| Ferric Chloride test | Absent | Present |
| Saponin | | |
| Foam test | Present | Absent |
| Test for Triterpenoids and Steroids | | |
| Salkowski's test | Present | Present |
| Libbermann-Burchard's test | Present | Present |

### 3.3 Quantitative Analysis

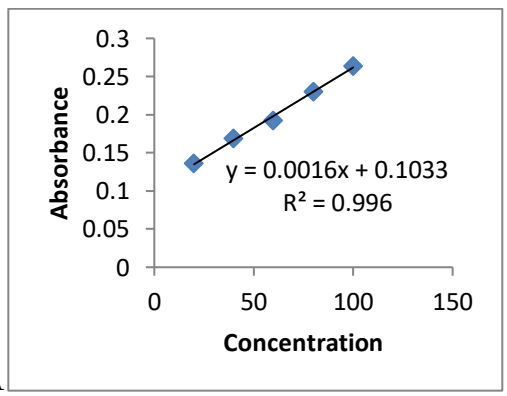
#### 3.3.1 Total Phenolic content (TPC) & Total Flavonoids content (TFC) estimation

**Table 4 Standard table for Gallic acid**

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.137 |
| 2. | 40 | 0.168 |
| 3. | 60 | 0.194 |
| 4. | 80 | 0.231 |
| 5. | 100 | 0.265 |

**Table 5 Standard table for Rutin**

| S. No. | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.165 |
| 2. | 40 | 0.195 |
| 3. | 60 | 0.258 |
| 4. | 80 | 0.297 |
| 5. | 100 | 0.319 |

A 

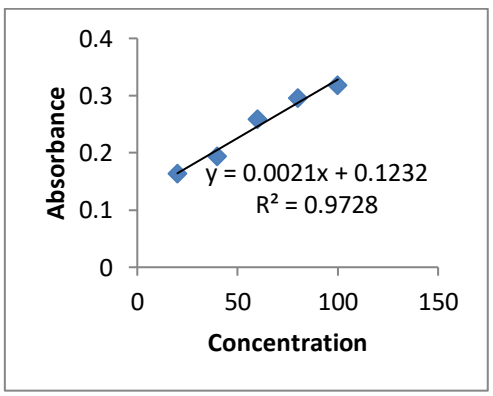

B 


**Figure 1: Graph represent standard curve of Gallic acid (A) and Rutin (B)**

### 3.3.1.1 Total Phenolic Content Total Flavonoid Content in extract

**Table 6: Total Phenolic Content**

| S.No | Absorbance | TPC in mg/gm equivalent of Gallic Acid |
|---|---|---|
| 1 | 0.145 | 62.05mg/gm |
| 2 | 0.169 | |
| 3 | 0.182 | |

**Table 7: Total Flavonoid Content**

| S.No | Absorbance | TFC in mg/gm equivalent of Rutin |
|---|---|---|
| 1 | 0.132 | 15.23 mg/gm |
| 2 | 0.153 | |
| 3 | 0.176 | |

### 3.4. Organoleptic properties

**Table 8: Organoleptic properties of Plant extract**

| Drug | Organoleptic properties | Observation |
|---|---|---|
| Extract | Colour | Reddish |
| | Odour | Characteristic |
| | State | Solid |

An evaluation of the plant extract organoleptic qualities, including color, odor, and state, was conducted. Plant extract was discovered to have a Dark reddish colour to it when tested. Extract has a Characteristic odor and has a solid state form, according to research conducted on it. Extract exhibited the same color, odor and state as the requirements for these characteristics. Result show in Table 8.

### 3.5 Solubility study

**Table 9: Solubility study of Extract**

| Drug | Solvents | Observation/Inference |
|---|---|---|
| Extract | Methanol | Freely Soluble |
| | Ethanol | Freely Soluble |
| | DMSO | Freely Soluble |
| | Water | Soluble |

The solubility of Extract was determined in various non-volatile or volatile liquid vehicles such as Dimethyl sulfoxide, methanol, ethanol, and water shown in Table 9. From the results, it was observed that the extract is freely soluble in Ethanol, Methanol and DMSO and soluble in water.

### 3.6 Evaluation parameter of formulations

### 3.6.1 Particle size determination and Zeta potential

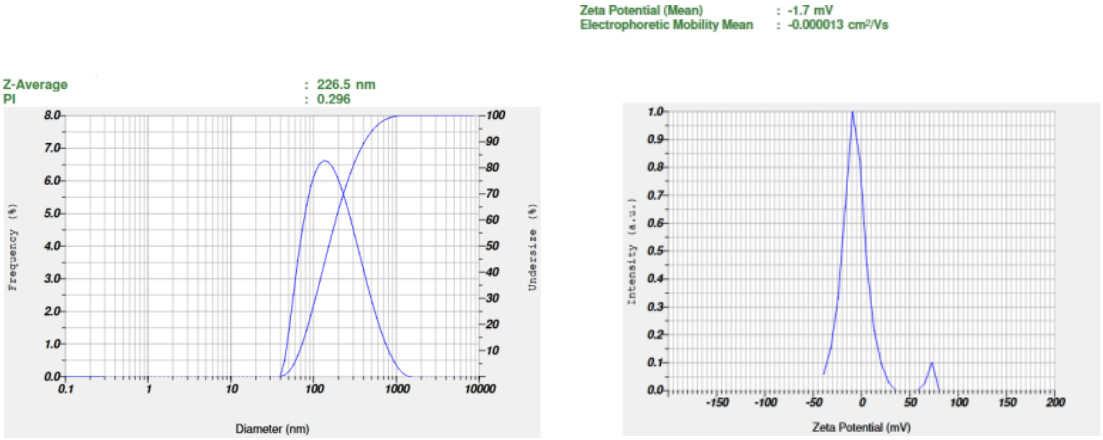



**Figure 2: Particle size and Zeta potential (F4)**

**Table 10: Result of Particle size and Zeta potential of all formulations**

| S.No. | Formulations | Particle size (nm) | PI Value | Zeta potential |
|---|---|---|---|---|
| 1. | F1 | 4458.2 nm | 6.373 | -7.5 mV |
| 2. | F2 | 1381.6 nm | 1.494 | -8.3 mV |
| 3. | F3 | 1396.4 nm | 0.593 | 4.5 mV |
| 4. | F4 | 1283.9 nm | 1.431 | -12.9 mV |
| 5. | F5 | 4199.6 nm | 8.280 | -1.7 mV |

The particle size is one of the most important parameter for the characterization of microsphere. The average particle sizes of the prepared microsphere formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of microsperes was found to be range between 1283.9 to 4458.2 nm. These particle size values indicate that the all formulated microsphere is under the range of microsphere and F4 is the lowest particle size of all formulation shown in above table 10. Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -1.7 to 4.5 mV with peak area of 100% intensity. These values indicate that the all formulated microsphere is stable.

### 3.6.2 Scanning electron microscopy characterization of F4 formulation

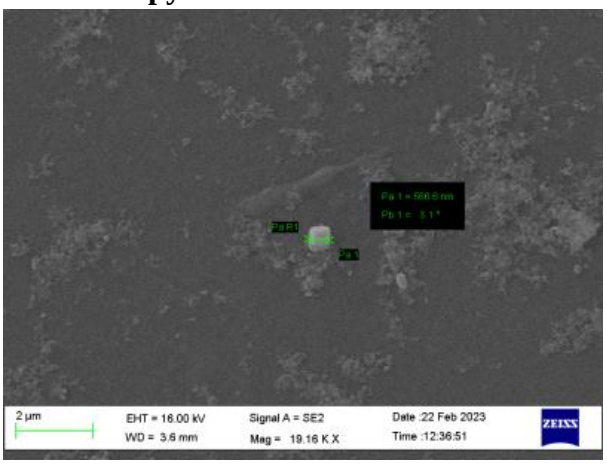



**Figure 3: SEM**

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared microsphere. Drug loaded microsphere were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared microsphere at 19.16 kx magnification showed that the microsphere were smooth surface morphology and spherical shape. The smooth surface morphology and spherical shape of microsphere was clearly observed in the SEM images. Show in above figure 3.

**3.7 Anti-microbial Activity (Well Diffusion Method)**

**3.7.1 Anti-microbial activity of *Pongamia pinnata* extract and formulation F4 against *C. albicans***

**Table 11: Anti-microbial activity of Pongamia pinnata against *C. albicans***

| **Samples** | **Zone of inhibition (mm)** |
|---|---|
| Plant Extract | 10 mm |
| Formulation (F4) | 16 mm |
| Standard (Fluconazole) | 20 mm |

**3.8 Stability study**

**Table 12: Stability Study of Microsphere (F4) formulation**

| **S.No** | **Time (Days)** | **$30^0$C±2 $^0$C and 60 ± 5% RH** | | | **$40^0$C±2 $^0$C and 70 ±5% RH** | | |
|---|---|---|---|---|---|---|---|
| | | **Appearance** | **Particle size nm** | **Zeta potential mV** | **Appearance** | **Particle size nm** | **Zeta potential mV** |
| 1. | 0 | Solid Powder | 1283.9 nm | -12.9 mV | Solid Powder | 1283.9 nm | -12.9 mV |
| 2. | 30 | Solid Powder | 1283.1 nm | -12.2 mV | Solid Powder | 1283.0 nm | -13.0 mV |
| 3. | 45 | Solid Powder | 1282.8 nm | -12.4 mV | Solid Powder | 1283.2 nm | -12.8 mV |
| 3. | 60 | Solid Powder | 1283.0 nm | -13.1 mV | Solid Powder | 1284.1 nm | -12.7 mV |
| 4. | 90 | Solid Powder | 1283.5 nm | -13.3 mV | Solid Powder | 1283.4 nm | -13.0 mV |

Formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions ($30^0$C±2 $^0$C and 60 ± 5% RH) and ($40^0$C±2 $^0$C and 70 ±5% RH). Physicochemical parameters, including appearance, Zeta potential and particle size were not altered significantly. Results of assay and other evaluation criteria at periodic time points of stability studies are summarized in Table 12.

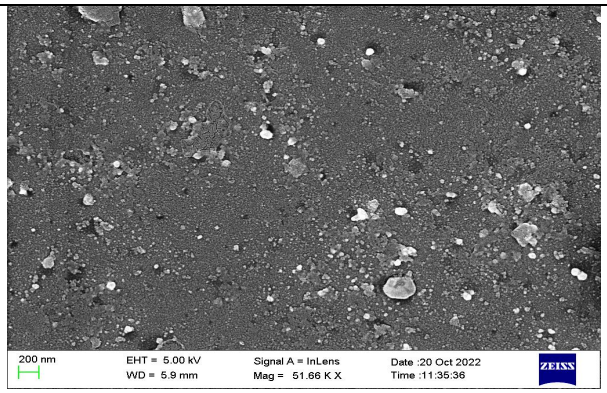



**Fig 4: showing the SEM analysis of the microspheres**

- ***In-vitro* Antimicrobial activity**

**Table 13: Antimicrobial activity of seed extract of Pongamia Pinata**

| S No. | Sample name | Zone of Inhibition (mm) |
|---|---|---|
| 1 | AgNO3 | 7mm |
| 2 | Extract | 12mm |
| 3 | Silver NPs | 17mm |

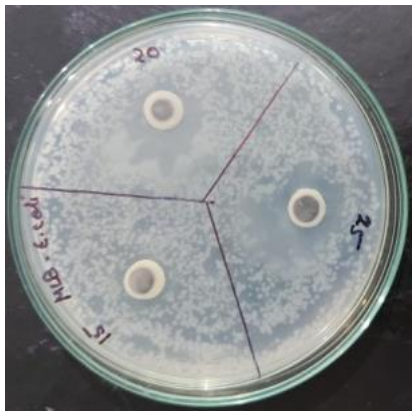



**Fig 5: showing the zone of inhibition of extract.**

## 4.CONCLUSION

Extensive work on the formulation and characterization of extract-loaded EC microspheres was evaluated. The micromeritics properties exhibited, that all microspheres were free-flowing in nature. SEM photographs confirmed the spherical shape of the microspheres. The results revealed that experimental conditions allowed a uniform distribution of the extract within EC microspheres having no significant effect on drug-polymer interaction. Finally, the results of this investigation elucidate that the process and formulation variables could be effectively altered to achieve the desired characteristics of the EC microspheres for novel delivery of the herbal drug.

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