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FORMULATION AND DEVELOPMENT OF MICROSPHERES LOADED GEL OF ANNONA SQUAMOSA EXTRACT FOR POTENTIAL ANTIMICROBIAL ACTIVITY

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ABSTRACT

Microspheres are small spherical Particles, with a diameter in the micrometer Range (typically 1 micrometre to 1000) micrometer Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres, and ceramic microspheres are commercially available Solid and Hollow microspheres Vary widely in Density and therefore, are used for different applications. Qualitative phytochemical screening of Annona squamosa showed the presence of active metabolites such as Alkaloids, flavonoids, glycosides, steroids, tannin and phenols is present. Formulation was carried out by Solvent Evaporation method. Trial batches indicated that polymers are suitable for the Annona squamosa microspheres. Polymer produced good formulations. HPMC and ethyl cellulose were selected for further studies. Scanning electron micrograph of the prepared microspheres at 1.00 kx magnification showed that the microspheres were smooth surface morphology and spherical shape. The spongy and porous nature of microspheres was clearly observed in the SEM images. Particle size and zeta potential was determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. Average particle size obtained for the formulations F1 and F5 were 143.0 to 171.5 nm. Zeta potential values of microspheres indicated that the formulated microspheres are stable. The amount of drug being entrapped in microspheres was calculated and all the prepared microspheres were found to possess very high entrapment efficiency.

INTRODUCTION

Novel drug delivery of herbal drugs

In the past few decades, considerable attention has been concentrated on the evolution of an NDDS for herbal drugs (Saraf 2010). Herbal drugs are getting more popular in the modern world for their diligence to cure a variety of diseases with less toxic effects and better therapeutic effects. Meanwhile, some limitations of herbal extracts/plant actives such as instability in highly acidic pH and liver metabolism have gone to attain the drug levels below to the therapeutic concentration in the blood resulting in less or no healing effect. Incorporation of novel drug delivery technology to herbal or plant actives minimizes the drug degradation or presystemic metabolism and serious side effects by accumulation of drugs to the nontargeted areas and improves the ease of administration in the pediatric and geriatric patients (Goyal et al., 2011). Conventional dosage forms, including prolonged-release dosage forms, are unable to fulfill the ideal requirements of novel carriers such as ability to deliver the drug at a rate directed by the penury of the body and to transmit the active entity of herbal drug to the site of activity. For good bioavailability, natural products must have a sound balance between hydrophilicity (for dissolving into the gastrointestinal fluids) and lipophilicity (to cross lipidic biomembranes). Many phytoconstituents such as polyphenolics have good water solubility, but are poorly absorbed (Manach et al., 2004) either due to their multiple-ring large-sized particles which cannot be soaked up by simple diffusion or referable to their poor miscibility with oil and other lipids, severely restricting their power to reach across the lipid-rich outer membranes of the enterocytes of the little bowel (Chauhan et al., 2009). Thus, the nano-sized NDDSs of herbal drugs have a potential future for enhancing the natural process and overwhelming problems related with plant medicines. Novel herbal drug carriers cure particular disease by targeting just the affected zone inside a patient's body and transporting the drug to that region. NDDS is advantageous in giving up the herbal drug at predetermined rate and delivery of drug at the site of action which minimizes the toxic effects with an increase in bioavailability of drugs. In novel drug delivery technology, control of the dispersion of the drug is achieved by incorporating the drug in carrier system or in modifying the social organization of the drug at the molecular level. Incorporation of herbal drugs in the delivery system also aids to increase in

solubility, enhanced stability, protection from toxicity, enhanced pharmacological activity, improved tissue macrophage distribution, sustained delivery, and protection from physical and chemical degradation. For example, liposomes act as potential vehicles to take anticancer agents by increasing amount of drug in tumor area and decrease the exposure or accumulation of drug in normal cells/tissues, thereby preventing tissue toxicity effects. The phytosomal carriers have been considered for effective delivery of herbal extracts of ginseng (Ginkgo biloba), etc. Direct binding of phosphatidylcholine to herbal extract components led to better absorption characteristics as compared to conventional delivery of herbal infusions. Other vesicular assemblies such as microspheres, animations, and polymeric nanoparticles have been shown beneficial to carry herbal components. The present review article is directed to supply an overview of different cases of drug delivery systems incorporating active ingredients and potential advantages of such organizations (Goyal et al., 2011). In the present study, an effort has been induced to touch on various aspects and applications related to novel herbal drug preparations.

Microspheres

To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects (Jain 1997). There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bioerodible polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones.

Microspheres are small spherical Particles, with a diameter in the micrometer Range (typically 1 micrometre to 1000) micrometer Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres, and ceramic microspheres are commercially available Solid and Hollow microspheres Vary widely in Density and therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Polyethylene and polystyrene microspheres are the two most common types of polymer microspheres. Polystyrene microspheres are typically used in biomedical applications due to their ability to facilitate Procedures such as Cell sorting and immune Precipitations **(Venkatesan *et al.*, 2009).**

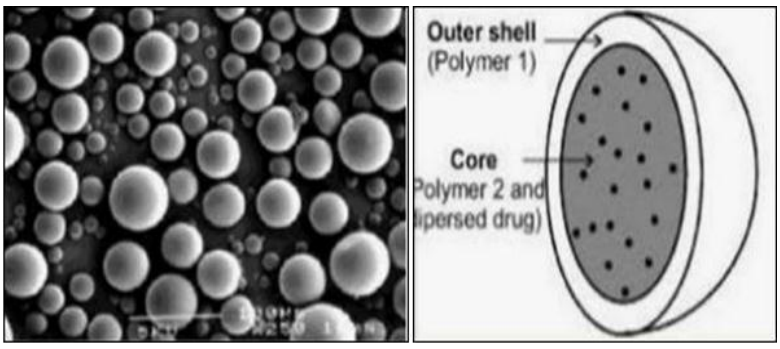


Figure 1: Microspheres.

Criteria for microsphere preparation

Incorporation of solid, liquid or gases into one or more polymeric coatings can be done by micro encapsulation technique (Ghulam et al., 2009). The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, coprecipitation etc.

Preparation of microspheres should satisfy certain criteria

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersability in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.

5. Biocompatibility with a controllable biodegradability
6. Susceptibility to chemical modification.

Types of microspheres

- Bioadhesive microspheres
- Magnetic microspheres
- Radioactive microspheres
- Floating microspheres
- Polymeric microspheres
- Biodegradable polymeric microspheres
- Synthetic polymeric microspheres

Characteristics of microspheres

1. Microsphere size may be critical to the proper function of an assay, or it may be secondary to other characteristics. Considering traditional diagnostic methods, the test or assay format commonly dictates particle size, such as the use of very small spheres (~0.1- 0.4μm) to ensure satisfactory wicking in lateral flow tests, or the use of larger, cell-sized spheres (~4-10μm) for beadbased flow cytometric assays.

2. Common microsphere compositions include polystyrene (PS), poly(methyl methacrylate) (PMMA), and silica. These materials possess different physical and optical properties, which may present advantages or limitations for different applications. Polymer beads are generally hydrophobic, and as such, have high protein binding abilities. However, they often require the use of some surfactant (e.g. 0.01-0.1% Tween® 20 or SDS) in the storage buffer to ensure ease of handling.

3. Microspheres may be coated with capture molecules, such as antibodies, oligonucleotides, peptides, etc. for use in diagnostic or separation applications. Microsphere coatings are typically optimized to achieve desired specific activity, while minimizing nonspecific interactions.

4. Many applications in the life sciences demand added properties, such as fluorescence or a visible color, or iron oxide inclusions for magnetic separations. Polymer spheres (and polymer based magnetic spheres) are often internally dyed via organic solvent swelling, and many standard products are available.

Advantages of microspheres

1. Decreased size of microsphere contributes increased surface area thereby increases the potency of the poorly soluble material (Kadam and Suvarna 2015).
2. Dose frequency and adverse effects can be reduced.
3. Increased patient compliance.
4. Drug packaged with polymer prevent drug from enzymatic cleavage therefore the drug can be protected from various enzymes.
5. Enhances bioavailability.
6. Gastric irritation can be reduced.
7. Biological half-life can be enhanced.

Limitation of microspheres

1. Some of the disadvantages were found to be as follows.
2. The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
3. The fate of polymer matrix and its effect on the environment.
4. The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
5. Reproducibility is less.
6. Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to be encapsulated.
7. The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents (Rina et al., 017).

Application of Microspheres

1) Microspheres in Vaccine Delivery
2) Microspheres in Gene Delivery
3) Oral Drug Delivery
4) Transdermal Drug Delivery
5) Targeting by Using Micro Particulate Carriers

Microspheres have been explored extensively for their use in the field of drug delivery and various polymers have been utilized for the formulation of the microspheres, which in turn have been assessed for different purposes. Microspheres are one of the multiple unit dosage forms. Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. Microspheres are potential drug delivery carrier systems in the segment of novel drug delivery and are prepared using assorted polymers. Polymer which is deacetylated derivative of (−4)2-acetamido-2-deoxy-b-d-glucose or chitin has been extensively explored for its various biomedical and pharmaceutical applications. Properties such as biodegradability, low toxicity, and good biocompatibility make it suitable for use in drug delivery and biomedical field. As a drug carrier, polymers have been investigated for the sustained delivery of many oral formulations and parenteral formulations**.** The main goal of the research work is development of microspheres loaded gel formulation of *Annona squamosa* (Leaves) extract for enhance stability as well as bioavailability of Phytoconstituents

Annona squamosa plant belonging to the family *Annonaceae* family.*Annona squamosa* leaves (ASLs) possess valorisation potential owing to their extensive pharmacological properties and biological activities, such as antioxidant, antimicrobial, antidiabetic, antiviral, anticancer, and hepatoprotective activities. The main

objective of the research work is to formulate microspheres containing extract of *Annona squamosa* leaves and later this formulation incorporate into a gel. The microspheres is selected for the *Annona squamosa* leaves extract in order to enhance its antimicrobial activity and microspheres encapsulated the plant extract and enhances the therapeutic value and also provide the stability to the extract also. In future it can be utilised easily converted into the conventional dosage form against the treatment of skin disorder.

Plant profile

Botanical name: *Annona squamosa Linn.*

Figure: ***Annona squamosal.***

Taxonomical classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Super-division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Magnoliales
Family: *Annonaceae*
Genus: Annona
Species: squamosa

Plant description

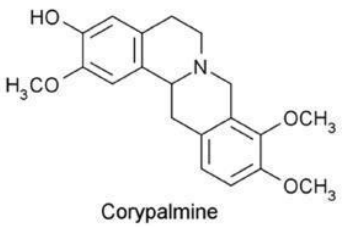
A. squamosa is an ever-green tree reaching 3–8 m in height. Leaf oblong lanceolate or lanceolate, 6–17 cm long and 3–5 cm wide, alternately arranged on short petioles; bark thin, gray; flower greenish, fleshy, drooping, extra-axillary, more on leafy shoot than on the older wood and tending to open as the shoot elongates; fruit can be round, heart-shaped, ovate or conical, 5–10 cm in diameter, with many round protuberance; seeds 1.3–1.6 cm long, oblong, smooth, shiny, blackish or dark brown (**Ma *et al.*, 2017)**.

Phytochemistry

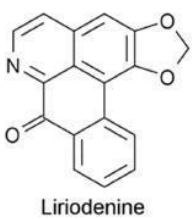
Phytochemical analyses of several parts of the *A. squamosa* plant have revealed the presence of a variety of phytochemicals and components, including diterpenes (DITs), alkaloids (ALKs), glycoside, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids, *annonaceous acetogenins* (ACGs), cyclopeptides (CPs) and essential oils. *Ananaceous acetogenins*, one of these, has emerged as a viable insecticide for agricultural and human usage (**Mondal *et al.*, 2018**).

Anonaine

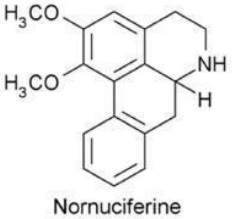
Asimilobine



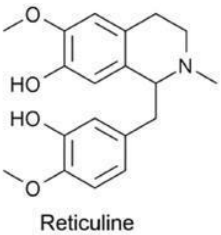
Corypalmine



Liriodenine



Nornuciferine



Reticuline

Pharmacological activity

- Anticancer activity
- Anti-Inflammatory Activity
- Anti-Oxidant Activity
- Anti-Bacterial Activity

MATERIAL AND METHODS

Plant collection

The medicinal plant *Annona squamosa* (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.

Authentication of selected traditional plant - Medicinal plant *Annona squamosa* was authenticated by a plant taxonomist (Dr. Jagrati Tripati. Govt. Botanist) in order to confirm its identity and purity. Plant Authentication no. AC/052/024.

Extraction of *annona squamosa*

The Soxhlet method is used to extract compounds with high thermic stability due to a high temperature (the boiling point of the solvent). These substances are concentrated during the extractive process. The material to be extracted is placed in a porous thimble placed in the extraction chamber, which is placed on a distillation flask in which the solvent to be heated is placed. As the liquid boils, its vapors rise along a side tube up to the refrigerant mounted on the extractor. The liquid obtained from the condensation of the vapors falls into the extraction chamber passing through the material contained in the porous thimble, filling it until it reaches the elbow of the lateral siphon. At this point, due to its weight, the percolated liquid is sucked into the underlying flask, from which it is distilled again. The cycle described above is repeated several times until the extraction is considered complete: in this way, it is possible to extract all the soluble material from the matrix always using the same volume of solvent previously loaded in the boiler, renewed continuously by the distillation process. (Naviglio et al., 2019)

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.

Figure: Continuous hot extraction.

Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used **(Kokate *et al.*, 2000).**

Formulation of microspheres by solvent evaporation method

Microspheres containing extract (*Annona squamosa*) as a core material were prepared by Solvent Evaporation method. Extract (*Annona squamosa*), HPMC and EC were dissolved in a mixture of ethanol and dichloromethane (1:1) at room temperature. 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 4: Composition of microsphere formulation.

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

Evaluation parameter of extract loaded microsphere

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. The size data is documented in Table 14**(Singh and Vingkar 2008).**

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. The zeta potential data is documented in Table 15. **(Đorđević*et al.*, 2022).**

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 **(Ahmed *et al.*, 2020).**

Formulation of microspheres loaded Gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and microspheres of optimized formulation (F1) was incorporated into the dispersion to obtained Gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps(**Abbas *et al.*, 2019, Silpa *et al.*, 2021**).

Table 5: Composition of gel formulation.

S. No.	Excipients	Quantity (gm)
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm
3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	Microspheres	1.0 gm
6.	Triethanolamine	q.s
7.	Water	100 ml

Characterization of microspheres loaded Gel

Physical appearance

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation **(Kumar and Eswaraiah 2020).**

pH

PH of the formulation was determined by using Digital pH meter (EI). The meter was allowed to stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper (**McGlynn, W. 2003).**

Viscosity

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 7 at 100 rpm at the temperature of 25^0C **(Monica and Gautami 2014).**

Spreadability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1 g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down. Spreadability was determined by the following formula

S= M*L/T

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T- Time for spreading gel in sec **(Sandeep, D. S. 2020).**

Skin irritation test

The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat 2-3 days before the experiment. The gel was applied on the properly shaven skin of rat. The animals were treated daily for 2-3 days, and undesirable skin changes, i.e., change in color, change in skin morphology was checked for a period of 24 h and erythema and edema on the treated skin were examined (**Giri *et al.*, 2019; Murthy *et al.*, 2001)**

Antibacterial Activity

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 121oC 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.

Well diffusion assay

The bacterial suspension of E. coli was standardized to 108 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 108 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate (Mohammadi et al., 2012). 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 microsphere, Microsphere loaded gel (1mg/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 37o 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 (Manandhar et al., 2019).

RESULTS AND DISCUSSION

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 *Annona squamosa* is shown in Table: 6

Table: Percentage Yield of crude extracts of *Annona squamosa* extract.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Annona squamosa*	Pet ether	300	1.35	0.47%
2		Methanol	284	6.58	2.31%

Preliminary phytochemical study table phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
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's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic compounds**		
6.1	Ferric Chloride test	Present	Absent
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present
8.2	Libbermann-Burchard's test	Present	Present

Evaluation parameter of microsphere formulations

Particle size determination

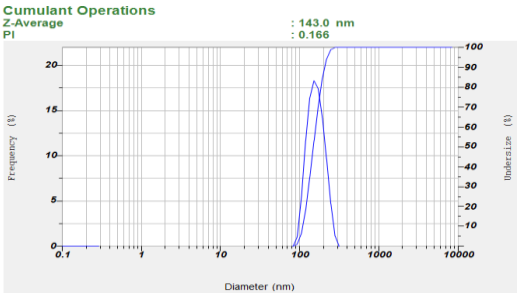


Figure: Particle size determination.

Result of Particle size of all formulations

S. No.	Formulations	Particle size (nm)	PI Value
1.	Microsphere (F1)	143.0 nm	0.166
2.	Microsphere (F2)	171.5 nm	0.365
3.	Microsphere (F3)	145.7 nm	0.290
4.	Microsphere (F4)	147.6 nm	0.269
5.	Microsphere (F5)	166.4 nm	0.322

DISCUSSION

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 microspheres was found to be range between 143.0 to 171.5 nm. These particle size values indicate that the all formulated microsphere is under the range of microsphere and F1 is the lowest particle size of all formulation shown in above table 14.

Zeta potential determination

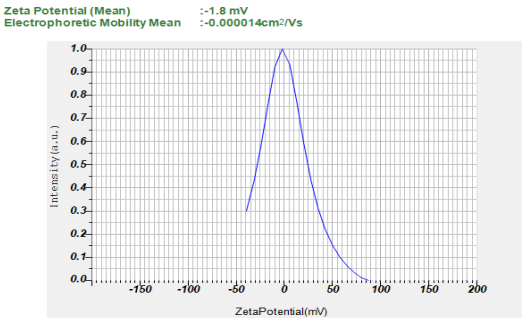


Figure: Zeta potential.

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 12.9 mV with peak area of 100% intensity. These values indicate that the all formulated microsphere is stable. Results show in above table.

Scanning electron microscopy characterization of F1 formulation

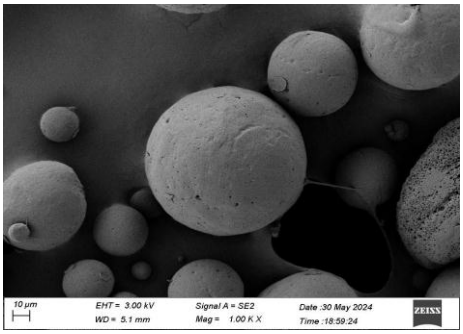


Figure: Scanning Electron Microscope (SEM).

DISCUSSION

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared microsphere. Extract 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 1.00 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 20.

Characterization of microsphere loaded gel

Physical appearance

Table 10: Physical appearance.

S. No.	Parameter	Result
1.	Colour	Green colour
2.	Odour	Odourless
3.	Appearance	Semisolid
4.	Homogeneity	Homogeneous

Discussion

An evaluation of the gel, including color, odor, appearance and homogeneity, was conducted. Gel was discovered to have a green color to it when tested. Gel does not have a distinctive odor and has a semisolid appearance, according to research conducted on it. Gel exhibited the same color, odor, and Appearance as the I.P. requirements for these characteristics.

Viscosity of gel

Table 11: Viscosity.

S. No	Formulation	Viscosity (cps)
1.	Gel	5990±0.53

Discussion

The viscosity was measured by the Brookfield viscometer spindle no. 7 at 100rpm. The result was shown in the table 17. The viscosity of Gel was found to be 5990 ±0.53 centipoise respectively.

pH determination

S. No.	Formulation	pH
1.	Gel	6.6

Discussion

The pH of the gel formulation was found to be 6.6, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time. The physicochemical properties of prepared gel formulation were in good agreement.

Spreadability

Table: Spreadability

S. No	Formulation	Spreadability (g.cm/s)
1.	Gel	11.01

Discussion

One of the essential criteria for a Gel is that it should possess good spreadability. Spreadability depends on the viscosity of the formulation and physical characteristics of the polymers used in the formulation. A more viscous formulation would have poor spreadability. Spreadability is a term expressed to denote the extent of area on which the gel readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends upon its spreading value. The spreadability of Gel formulation is found to be 11.01 g.cm/s.

Results of antimicrobial activity of formulation
Antimicrobial activity of Formulation against *E.coli*

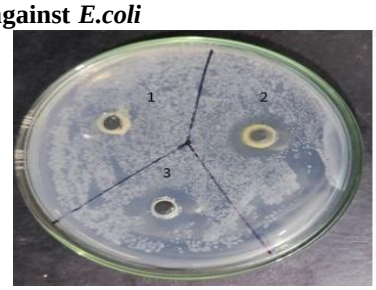


Figure 19: Antimicrobial activity.

Table: Antimicrobial activity of Formulation and plant extract against *E.coli*

S. No.	Sample Name	Zone of Inhibition (mm)
1.	Extract	6 mm
2.	Microsphere gel (0.5mg/ml)	12 mm
3.	Microsphere gel (1mg/ml)	17mm

SUMMARY AND CONCLUSION

Qualitative phytochemical screening of *Annona squamosa* showed the presence of active metabolites such as Alkaloids, flavonoids, glycosides, steroids, tannin and phenols is present. Formulation was carried out by Solvent Evaporation method. Trial batches indicated that polymers are suitable for the *Annona squamosa* microspheres. Polymer produced good formulations. HPMC and ethyl cellulose were selected for further studies. Scanning electron micrograph of the prepared microspheres at 1.00 kx magnification showed that the microspheres were smooth surface morphology and spherical shape. The spongy and porous nature of microspheres was clearly observed in the SEM images. Particle size and zeta potential was determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. Average particle size obtained for the formulations F1 and F5 were 143.0 to 171.5 nm. Zeta potential values of microspheres indicated that the formulated microspheres are stable. The amount of drug being entrapped in microspheres was calculated and all the prepared microspheres were found to possess very high entrapment efficiency.

The viscosity of microsphere loaded gels found to 5990±0.53cps. The pH of microsphere loaded gel is 6.6 and spreadability is 11.01, indicating that microsphere loaded gel has high release and permeability.

The formulation and characterization of microspheres loaded gel was performed in the present research work. SEM photographs confirmed the shape and formation of the microspheres. The results revealed that experimental conditions allowed a uniform distribution of the extract in microspheres having no significant effect on drug-polymer interaction. Finally, there results of this investigation elucidate that the process and formulation variables could be effectively altered to achieve the desired characteristics of the microspheres loaded gel for novel delivery of the extract.

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