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FORMULATION AND CHARACTERIZATION OF ANNONA SQUAMOSA LINN LEAF EXTRACT NANOSUSPENSION FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

Currently, nanodelivery systems are an area of intense focus for the delivery of bioactive compounds to ensure effective treatment of various chronic diseases. *Annona squamosa* L. has belonging to the family Annonaceae and the leaves have shown to possess larvicidal, hepatoprotective, anti-bacterial, wound healing, anti-diabetic, etc,. Methanolic extract of *Annona squamosa* L. leaves, a poorly water-soluble drug is formulated as nanosuspension by nanoprecipitation method using Tween 80 and Span 80 as polymers in different ratio, for the improvement of solubility and bioavailability. The *Annona squamosa* L. nanosuspension were characterized by particle size, zeta potential, drug entrapment efficiency are 223.2nm, -12.4mV, 81% respectively. *In vitro* drug release and drug kinetics were also studied. The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time vs Concentration. It was concluded that *Annona squamosa* L. nanosupension facilitate higher cellular penetration and possess high bioavailability and sustained release.

KEYWORDS: Nanosuspension, *Annona squamosa* L., Nanoprecipitation, Solubility and bioavailability.

INTRODUCTION

Herbal medicines are extensively used all over the world since ages. Many phytomedicines, due to their poor absorption, exhibit least or no considerable *in vivo* activity despite of their amazing *in vitro* potential. It has been extensively suggested to incorporate herbal drugs with nanosuspension technology.

Nanosuspensions are sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster. The reduced particle size renders the possibility of administration of poorly soluble drugs like oral, topical, parenteral, ocular and pulmonary routes. The suspensions can also be lyophilized and into a solid matrix.[1,2]

METHODOLOGY

Collection and drying

Healthy plant leaves of *Annona squamosa* L. were collected from nearby farm. The leaves were authenticated by Botanical Survey of India, Coimbatore. The leaves were cleaned properly in running tap water and were shade dried. It was powdered in a mechanical mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

Collected leaves ***Shade dried leaves***

Figure 1: Collection and drying of Annona squamosa L. leaves.

***Preparation of methanolic extract of Annona squamosa* L.**

About 500g of the dried powdered leaf of *Annona squamosa* L. was defatted with 1.5L petroleum ether (60-800^{0}C) by maceration. The solvent was removed by filtration and the marc was dried. The marc was further extracted with methanol (95%) for 6 hrs in Soxhlet apparatus. Then the methanolic extract was concentrated by Rotary Evaporator for about 3 hrs at 40°C. The extract was dried under vacuum oven.

Figure 2: Soxhylation Process.

Preparation of Annona squamosa nanosuspension

Nano-precipitation method was followed with a slight modification for the preparation of nanosuspensions. *Annona squamosa* L. leaf extract (20mg), Polyethylene glycol and Sodium lauryl sulphate was dissolved in 2 ml of acetone by sonication for 60 seconds. The resultant solution was then gradually injected (1 ml min^{-1}) with a syringe connected to a thin teflon tube, into 20 ml water containing Span 80 with continuous magnetic stirring at 1000 rpm for 20mins. The resulting emulsion obtained was then diluted in 50 ml PVA solution (0.2% w/v in water) in order to minimize coalescence and the mixture was continuously stirred (500 rpm) for 6 h at room temperature to allow solvent evaporation and nanoparticle formation. The resultant nanosuspension was kept under vacuum at 25^{0}C for 2 hrs to remove organic solvents.

Characterisation of Nanosuspension[8]

Fourier Transformed Infrared (FTIR) Spectroscopy

FTIR spectroscopy was conducted using a Shimadzu FTIR 8400 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000–400 cm^{-1}. The procedure consisted of dispersing sample (drug, stabilizer, physical mixture) by KBr pellet method. The pellet was placed in the light path and the spectrum was recorded.

Particle Size and PDI

Mean particle size and size distribution (polydispersity index) of the prepared nanosuspension was determined by using Zetasizer [Zetatrac, Microtrac, Japan] which follows the principle of light diffraction, also called Photon Correlation Spectroscopy (PCS). Prior to the measurement, the samples were appropriately diluted with water to a suitable scattering intensity and re-dispersed by shaking before measurement.

Zeta Potential

The Zeta potential is a measure of the electric charge at the surface of the particles, indicating the physical stability of colloidal systems. The zeta potential values higher than |30mV| indicate long-term electrostatic stability of aqueous dispersions. In this study, the Zeta Potential was assessed by determining the electrophoretic mobility of the particles using Zetasizer [Zetatrac, Microtrac, Japan].

Percentage Entrapment Efficiency

In order to determine the % entrapment, around 2ml of formulation were taken in the Nessler's cylinder tube (10ml). The solution was centrifuge in the centrifuge machine at 2000-3000 rpm for 4 hrs. The supernatant layer was filter through whatmann filter paper number 41 and diluted with phosphate buffer 7.4 up to 10 ml and the resultant solution were analysed at particular wavelength of drug in nm using UV Double beam Spectrophotometer-3650.

These was carried out for three time and the result were calculated. The Percentage entrapment efficiency was calculated according to the equation or formula.

$$\%EE = \frac{\text{Total drug content - Free dissolve drug} \times 100}{\text{Drug amount used}}$$

INVITRO DRUG RELEASE STUDIES

Nanosuspension preparation was taken in dialysis membrane of 5cm length and suitability suspended in beaker containing 200ml of dissolution medium (phosphate buffer saline pH 7.4). The medium was maintained at temperature of 37-37.5^{0}C. It was stirred by means of magnetic stirrer at a constant speed. Sample of 1ml (diffusion medium) was withdrawn at every 1hr for 24hrs and replaced the diffusion medium. So that the volume of diffusion medium was maintained constant at 200ml. The sample were measured spectrophotometrically at 662nm.

KINETIC STUDIES

Zero-order release

$Q = Q_0 + K_0t$

Where,

Q - amount of drug dissolved at particular time 't'

Q_0 - initial amount of drug in solution at t = 0

K_0 - Zero-order release constant

First-order release

$\log Q_t = \log Q_0 + K_t / 2.303$

Where,

Q = Amount of drug dissolved in time 't'

Q_0 = Initial amount of drug in the solution

K_t = First order release constant

Higuchi Model
$Q = k.t_{1/2}$

Korsmeyer-Peppa's model
$Q = Kt^n$
Taking log on both sides of equation,
log Q = log K + n log t
Where,
Q = Cumulative % of drug release
t = Time
n = slope of linear plot of log Q Vs log t

RESULTS

Phytochemical Studies

The Methanolic Extract of *Annona squamosa* L. leaves was subjected to phytochemical studies to find out the presence and absence of constituents.

Table 1: Phytochemical analysis of methanolic extract of *Annona squamosa* L.

S.No.	Secondary Metabolites	Methanolic Extract Of *Annona squamosa* L.
1.	Steroids	+
2.	Alkaloids	+
3.	Flavanoids	+
4.	Saponins	-
5.	Glycosides	+
6.	Phenols	+

FOURIER TRANSFORMED INFRARED (FTIR) SPECTROSCOPY

Table 2: FTIR interpretation.

FREQUENCY	GROUP ASSIGNED
3322.16 cm^{-1}	OH Stretching
1690.53 cm^{-1}	Ar-C=O Ketone
2832.27 cm^{-1}	C-H Stretching (Methyl)
1252.68 cm^{-1}	C-O Stretching
1108.99-978.81 cm^{-1}	C-C Stretching
794.62 cm^{-1}	R_2C=CHR Trisubstituted

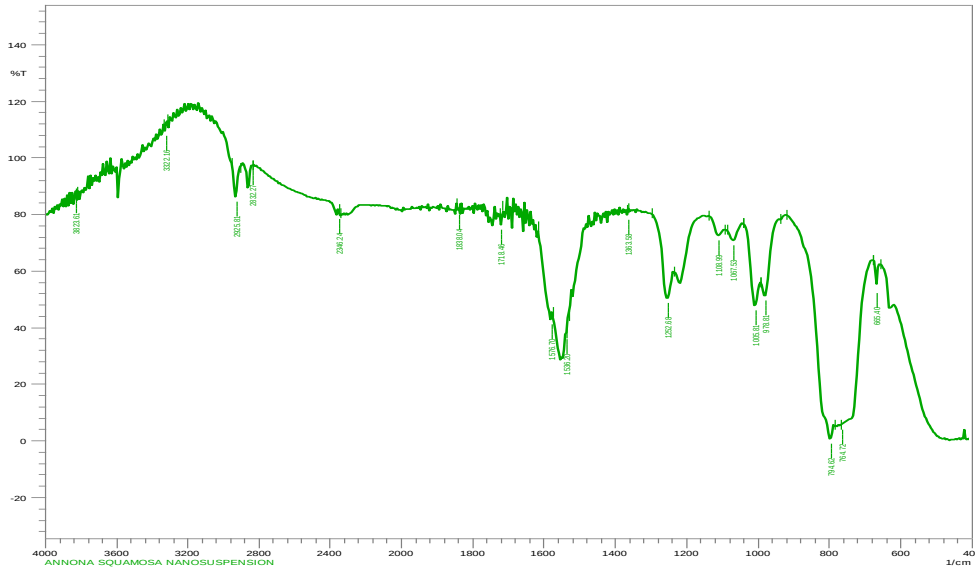


Figure 3: FTIR of Annona squamosa L. Leaf extract Nanosuspension.

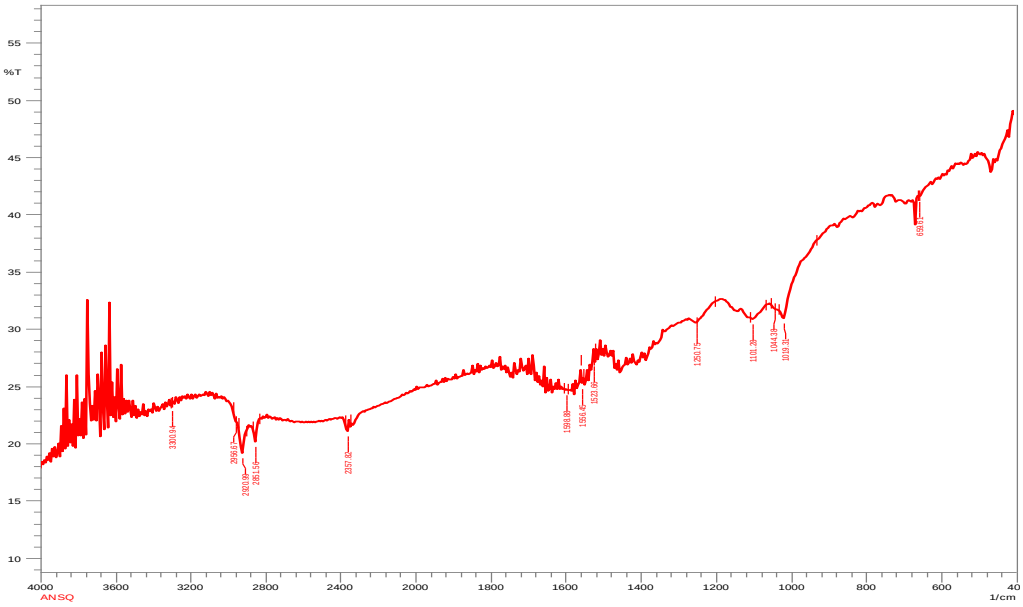


Figure 4: FTIR of Annona squamosa L. leaves extract.

FTIR spectroscopy was carried out to study the compatibility of pure *Annona squamosa* with the excipients used in the formulation of nanosuspensions. The methanolic extract of *Annona squamosa* has characteristics IR peaks at 3300.01 cm^{-1}, 2920.99 cm^{-1}, 2357.82 cm^{-1}, 1250.75 cm^{-1}, 1101.28 cm^{-1}, 1019.31 cm^{-1}, 659.91 cm^{-1}. The physical mixture of PEG+SLS, Span 80 and Tween 80 has characteristic IR peaks at 2823.59 cm^{-1}, 2854.45 cm^{-1}, 2357.80 cm^{-1}, 1252.68 cm^{-1}, 1108.99 cm^{-1}, 794.62 cm^{-1}, 764.72 cm^{-1}. The Annona squamosa nanosuspension has characteristic IR peaks at 3322.16 cm^{-1}, 2823.27 cm^{-1}, 1718.46 cm^{-1}, 1363.58 cm^{-1}, 1252.68 cm^{-1}, 1108.99 cm^{-1}, 1067.53 cm^{-1}, 1005.81 cm^{-1}, 978.81 cm^{-1}, 794.62 cm^{-1}, 764.72 cm^{-1}, 665.40 cm^{-1}.

FTIR spectroscopy reveals that there is no interaction between drug and excipients.

PARTICLE SIZE AND PDI

Particle size of optimised formulation is **223.2nm** and PDI is **0.277** as the smaller particle size may in turn bring about more bio-availability.

ZETA POTENTIAL

The reduced zeta potential values of **-12.4mV** indicated that the prepared nanosuspension possess a higher degree of long-term stability.

ENTRAPMENT EFFICIENCY

Table 3: Entrapment Efficiency Of *Annona Squamosa* L. Nanosuspension.

FORMULATION CODE	ENTRAPMENT EFFICIENCY
ASNF1	69%
ASNF2	72%
ASNF3	75%
ASNF4	80%
ASNF5	79%
ASNF6	74%
ASNF7	78%
ASNF8	81%

The entrapment efficiency of the optimised formulation was formed to be **81%**

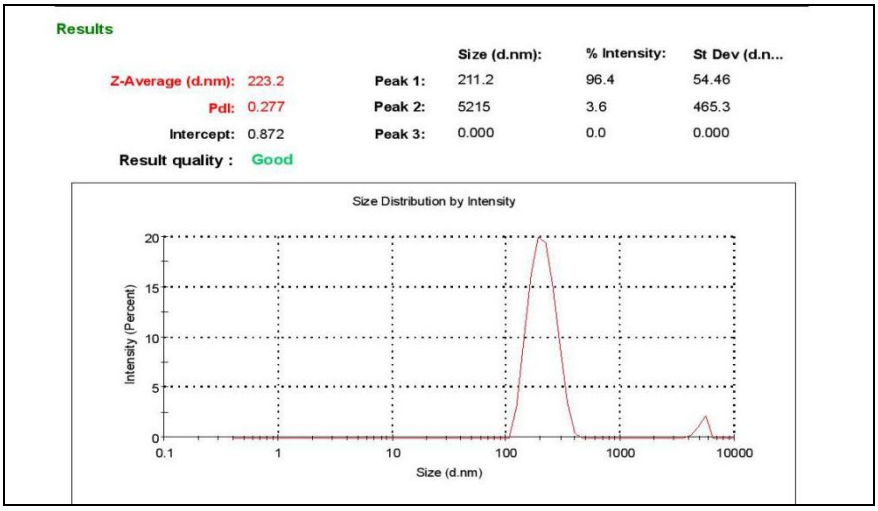


Figure 5: Particle size of Annona squamosa L. nanosuspension.

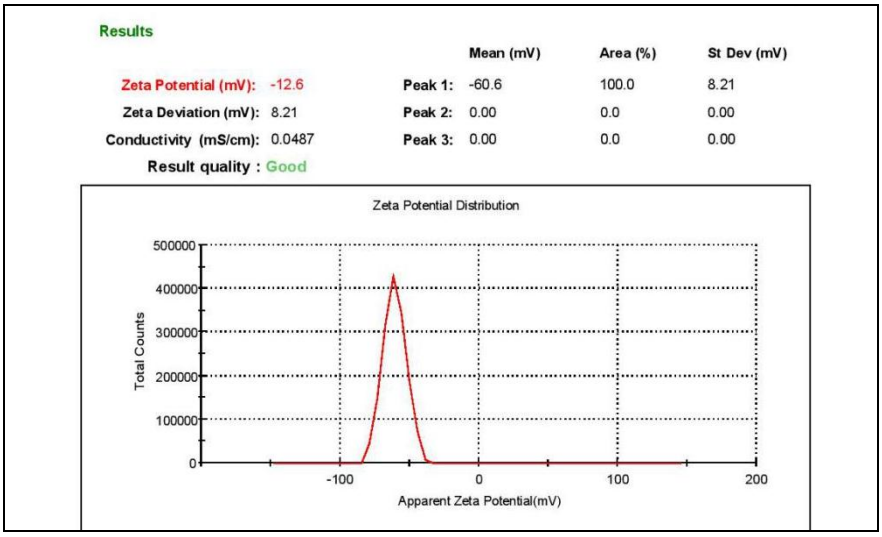
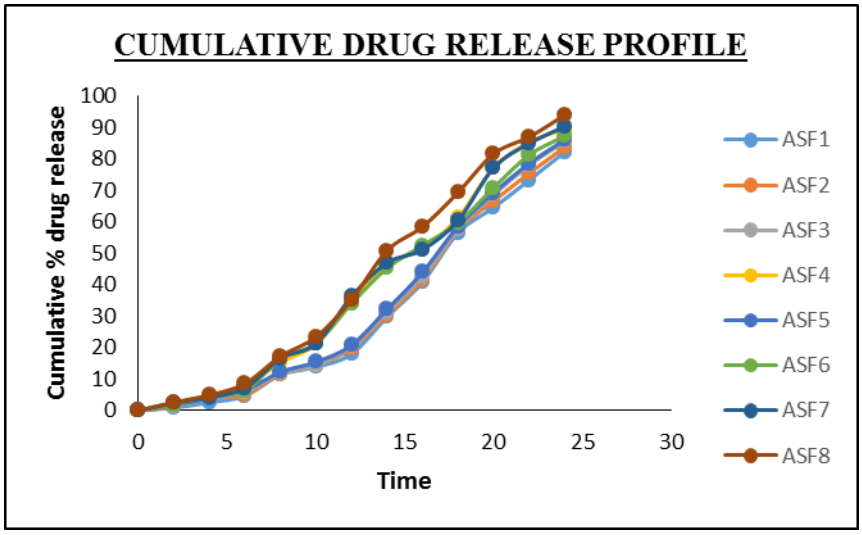


Figure 6: Zeta Potential of Annona squamosa L. nanosuspension.

INVITRO DRUG RELEASE STUDIES

Table 4: Cumulative profile for different formulations of *Annona squamosa* L. Nanosuspension.

TIME	% CUMULATIVE DRUG RELEASE							
	ASNF1	ASNF2	ASNF3	ASNF4	ASNF5	ASNF6	ASNF7	ASNF8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.92	1.19	1.41	2.32	1.67	1.86	2.41	2.65
4	2.54	3.85	3.67	4.18	3.98	4.52	4.28	4.92
6	4.67	5.12	5.88	7.57	6.45	6.25	7.13	8.53
8	11.34	11.66	11.83	15.22	12.27	16.51	16.57	17.22
10	13.85	14.29	14.29	21.14	15.43	21.39	21.29	23.38
12	17.97	19.81	20.44	34.26	20.82	33.98	36.23	35.17
14	29.76	30.43	31.13	46.32	32.17	45.22	46.95	50.82
16	40.81	41.56	42.54	51.45	43.98	52.49	51.12	58.41
18	56.49	57.4	57.71	61.30	58.51	59.63	60.44	69.32
20	64.51	66.65	68.58	77.12	69.18	70.82	77.12	81.55
22	73.22	75.42	77.92	85.27	78.52	81.23	84.85	86.92
24	82.14	83.70	85.56	89.45	85.96	87.25	90.25	93.87



At the end of the 24 hours, the invitro release of the diferrent formulations were found to be in the range of **82.14% - 93.87%.** The release of drug from the formulations prepared by Span 80 is less compared to that of Tween 80.

The reason for more release from the nanosuspension formulated with Tweens because of high HLB value compare to that of Spans.

Solubilisation will be more as the concentration of surfactants also increases. From the above invitro release table the optimised formulations were found to be F8 because of higher invitro release of **93.87%** when compared to the other formulations. As it is more hydrophilic it enhances the release of the drug.

INVITRO KINETIC STUDIES

Table 5: Regression value of optimum formulation ASNF8.

Formulation code	Regression value (r^2)		
	Zero order	Higuchi	Korsmeyer Peppa's
ASNF8	0.9821	0.9784	0.9837

CONCLUSION

In this study we have taken an effort to prepare nanosuspension formulation of *Annona squamosa* L. and the vesicles formed are quite stable. FTIR analysis indicates that there was no interaction. From the results of the present experimental investigation, it may be concluded that formulation of *Annona squamosa* L. nanosuspension showing small vesicle size, high percentage of entrapment with desire release of *Annona squamosa* L. Hence, **ASNF8** is the optimized formulation. The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time vs Concentration. So, we can conclude that *Annona squamosa* L. nanosupension facilitate higher cellular penetration and possess high bioavailability and sustained release.

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