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Formulation and Development of Nanoparticles of Quassia Amara for Treatment of Diabetes

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ABSTRACT: The green synthesis of nanoparticles uses plant components as reducing agents to create silver nanoparticles, including carbohydrates, lipids, enzymes, flavonoids, terpenoids, polyphenols, and alkaloids. The most common cases reported in all over world are those related to diabetes mellitus. Type 2 diabetes makes up about 90% of cases of diabetes, with the other 10% due primarily due to type 1diabetes mellitus and gestational diabetes. Green synthesis of nanoparticles provides nanomaterial benefits ranging from antidiabetic properties to natural reducing properties and stabilizing properties. Due to its insolubility in water, the diabetic drug *Quassia amara* L. has a poor bioavailability. The purpose of this study is to increase solubility of drug by formulating novel *Quassia amara* L. nanoparticles using the solvent evaporation method; thus, enhancing bioavailability. Drug and excipient compatibility study was carried out by FTIR. The prepared formulations were evaluated for visual examination, percentage yield, drug entrapment efficiency, drug content, FTIR, particle size, polydispersity index, zeta potential, SEM, *In-vitro* drug release, kinetic assessment study and *In vitro* antidiabetic study. The optimized formulation shows particle size 148.5nm. The zeta potential was found to be -28mV while Entrapment efficiency was found to be 92.5%. Maximum drug release can be confirmed through in-vitro testing. i.e., 82.99 % in 8hr. comparison of alpha-amylase inhibition of the acarbose and *Quassia amara* L. nanoparticles shows anti-diabetic activity.

Keywords: Nanoparticles, *Quassia amara* L., Diabetes, Poly-dispersity index, Zeta potential, Entrapment efficiency.

INTRODUCTION

Nanotechnology is the fastest growing field in the area of interdisciplinary research, especially in biotechnology (Natarajan *et al*., 2010) Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles-based therapeutics have been approved clinically for infections, vaccines, and renal diseases. (Narayanaswamy *et al.*, 2015). A nanoparticle can range in size from 1 to 100 nm (Konwar and Ahmed 2013). A drug may be connected, dissolved, trapped, or encapsulated within a nanoparticle matrix. Nanospheres and Nano capsules are the two different forms of nanoparticles (Noel and Tatenda 2020). *Quassia amara* L. also known as *Quassia Amarga*, belongs to the Simaroubaceae family, *Quassia amara* L. is a herb used for reducing blood glucose level from ancient time (Ferreira, 2013). The drug is presently given as an oral formulation, tablet in the dose of 390 mg twice daily and also in the form of capsule 500 mg (Acharya Balkrishna, 2021). In traditional medicine people used to keep the bark in water for overnight and drink it in early morning to reduce blood glucose level (Remya, 2017). Green synthesis of nanoparticles is a kind of bottom-up approach where the main reaction occurring is reduction/oxidation (Bhosale *et al.*, 2014). The biosynthesis of nanoparticles by physical and chemical processes was costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical application. Green synthesis method did not use any chemicals for reducing metal ions. So, the resultant product is more compatible and cost effective and no need to use high temperature, energy and pressure (Shanker *et al.*, 2015). Many conventional dosage forms are available for the treatment of diabetes mellitus type 2 like capsule, tablet. Quassia is used to treat stomach and diabetes. To avoid the breakdown of metabolic hormones like GLP-1 by the enzyme DPP-IV, which primary physiologic function in diabetes. (Ezekiel, 2022). In the current study, an effort was

made to create a novel drug delivery system using *Quassia amara* L. nanoparticles that reduce side effects and provide site-specific action (Pawar *et al*., 2022).

MATERIAL AND METHODS

Material. *Quassia amara* L. bark purchased from Dagdu Teli, Nashik. Silver nitrate was purchased from alpha chemika, Mumbai.α-amylaseenzyme, 3,5-Dinitrosalicylicacid, Sodiumchloride purchased from Himedia, Mumabai. Sodium potassium tartrate, Starch, Sodium hydroxide, Sodium phosphate monobasic from Modern science, Nashik.

Experimental Work

I. Pre- formulation study

Characterization of Drug

A. Organoleptic Properties of *Quassia amara* L.

Color of the drug sample of *Quassia amara* L. were studied by using a visual method. Very small quantity of *Quassia amara* L. was smelled to get the odor. Appearance was observed by visual method. The results of organoleptic properties of *Quassia amara* L. shown in Fig. 1 and Table 1.

B. Melting Point:

The melting point of *Quassia amara* L. was determined by placing a small amount of the substance in a sealed capillary tube with one end closed, the tube in the Thieles tube, with a thermometer fastened with a rubber band. The heating allows for the detection of the temperature ranges at which the sample melts. The sample's melting point coincides with its freezing point; hence the temperature stays constant while heating. The result shown in Table 2 (Supriya *et al.*, 2018).

C. **Solubility study:**

The solubility of a drug is determined by the shake flask method. The drug was dissolved in (water, acetonitrile and DMSO) 10ml of solvent. The solution was then agitated for 8 hours at 37°C using a magnetic stirrer to help it equilibrate. The material was taken out after 8 hours, filtered, and examined with a UV spectrophotometer (ShimadzuUV-2600). The results shown in Table 3 (Mehnert and Mäder 2001).

D. Determination of λ_{max} by UV Spectrophotometer Method:

Quassia amara L. UV spectrum was obtained using a UVS himadzu 2600 in Japan. The accurately weighed 10 mg *Quassia amara* L. was dissolved in a sufficient amount of Phosphate buffer pH 7.4 and the volume was increased to10ml. Stock solution was diluted to get a 100 ug/ml concentration. The 1 ml of aliquot was with drawn and the volume was made up to 10 ml by using Phosphate buffer pH 7.4 to obtain a 10 ug/ml concentration. The resultant solutions were scanned from 200 to 800 nm. The spectrum was recorded to get a reading of the maximum wavelength. The Fig 2. shows the scanned spectrum (Pawar *et al*., 2022).

Calibration curve of *Quassia amara* L. in Phosphate buffer pH 7.4

The stock solution of 100ug/ml was prepared in Phosphate buffer pH 7.4. This stock solution was used to prepare different dilutions in the range of 4-20ug/ml. AUV visible spectrophotometer was used to measure the absorbance of the resulting solutions at 220nm. Calibration curve shown in Fig. 3 (Pawar *et al*., 2022).

Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectrum of pure *Quassia amara* L. and silver nitrate was recorded by SHIMADZU 8400 FT-IR spectrometer. Sample was prepared by mixed with spectral grade KBr (3 mg sample in 100 mg KBr) and pressed into discs under hydraulic pressure. FTIR spectra were recorded in the range 4000-400 cm-1. the measured peaks in the spectrum observed in FT-IR. The results shown in Fig. 4, 5 and Table 4, 5 (Holler, edition: 6).

II. Synthesis of Nanoparticles

Preparation of Stock Solution

30g of dried aqueous extract was weighed and diluted to 10 ml with distilled water.

Preparation of 1mM silver nitrate aqueous solution

0.0167g of silver nitrate was dissolved in 1000 ml of distilled water and stored in amber coloured bottle until further use (Akshada *et al*., 2023).

Preparation of batches

Silver nitrate was utilized in varying concentrations to create the nanoparticle formula. Total 4 batches were prepared for formulation. Batches are shown in Table 1 (Shakeel *et al*., 2016).

Table 1: Formulation of *Quassia amara* L. Nanoparticles

Sr. No.	Ingredient	F1	F2	F3	F4
1.	*Quassia amara* L. Extract	30 mg	30 mg	30 mg	30 mg
2.	Silver nitrate (1 mM)	25 ml	50 ml	75 ml	100 ml

Quassia amara L. Nanoparticle were prepared by solvent evaporation method. The formula of nanoparticles was prepared by using different concentration of silver nitrate.

Synthesis Of *Quassia amara* L. Nanoparticles

In an Erlenmeyer flask, a 1 mM solution of silver nitrate was prepared. Then integrated 25ml, 50ml, 75ml, and 100 ml silver nitrate solution in 10 ml of *Quassia amara* L. aqueous extract was taken in a beaker (4 batches) and paced on a magnetic stirrer. To this 25 ml, 50 ml, 75 ml, and 100ml (1mM) silver nitrate solution was integrated drop wise with constant stirring at 3000rpm. The colour change of the solution was checked periodically (Shakeel *et al*., 2016).

Separation of Nanoparticles

The synthesised *Quassia amara* L. nanoparticles were dissevered by centrifugation utilizing REMI centrifuge at 3500 rpm for 3 Hr. The supernatant liquid placed in Petri plate and kept it on hot air oven for evaporation and nanoparticles were accumulated in powder form (Sadeghi *et al.*, 2015).

Evaluation of Nanoparticles

(a) Visual Examination of Nanoparticles

The primary confirmation of the synthesised *Quassia amara* L. silver nanoparticles is done by visual basis. The colour change of *Quassia amara* L. extract and silver nitrate solution with respect to time was observed. Fig 6 and Table 7.

(b) Percentage Yield of Nanoparticles

Regarding drug and excipient weight, the yield of nanoparticles was assessed. Nanoparticles are prepared, collected, dried, and weighed using an electronic

balance. The % yield has been calculated using a method. The percentage yield of nanoparticles was measured by using following formula. The results are appeared in Table 8 (Latha and Razia 2021).

Percentage yield = Weight of Nanoparticles/Weight of Excipient + Weight of drug× 100

(c) Drug Entrapment Efficiency

10 mg drug of *Quassia amara* L. nanoparticles were dissolved in Phosphate buffer pH 7.4. After centrifugation amount of drug present in supernatant was determined by UV spectrophotometer at 220 nm. The result appears in Table 9 (Das *et al*., 2005).

% Entrapment efficiency

$$= \frac{\text{Total drug loaded} - \text{Unentrapped drug}}{\text{Total drug loaded}} \times 100$$

d) Drug content

By weighing nanoparticles equal to 10 mg of *Quassia amara* L. and dissolving them in 100 ml of phosphate buffer 7.4, one can measure the number of drugs in each formulation. Serial dilutions were carried out until undissolved matter was filtered via Whatman No. 1 filter paper. Absorbance was measured at 220nm. The results appear in table 10 (Lakshmana Prabu *et al*., 2009).

(e) FT-IR Spectroscopy of Formulation (F4)

FTIR spectrophotometer was used to record the FT-IR spectrum of formulation. The formulation batch contained KBr, and the spectrum was conducted using KBr as a blank, yielding results in the 4000–400 cm^{-1} KBr range. The results appear in Fig. 7 and Table 11 (Holler, sixth edition).

(f) Particle Size Measurement

The dried powders of *Quassia amara* L. silver nanoparticles dispersed in water to obtain proper scattering intensity *Quassia amara* L. of silver nanoparticles. The particle size was determined by Malvern zeta size analyser. Results observed in Fig. 8 (Supriya *et al.*, 2018).

(g) Poly-dispersity index:

PDI is an index of width or spread or variation within the particle size distribution. Monodisperse sample have lower PDI value, whereas PDI of higher value indicate a wider particle size distribution and polydisperse nature of sample. The result appears in Fig. 8 (Supriya *et al.*, 2018).

(h) Zeta Potential Measurement

The Zeta Sizer (Malvern Instruments) was used to measure the zeta potential. It has zeta cells, polycarbonate cells with gold-plated electrodes, and uses water as the sample preparation medium. The silver nanoparticle's surface potential is determined by the zeta potential, which is essential for characterising the stability of nanoparticles. Results appear in Fig. 9 (Supriya *et al.*, 2018).

(i) Scanning Electron Microscopy

The morphology of vesicles was examined using a scanning electron microscope (SEM). SEM analysis of *Quassia amara* L. nanoparticles were performed to evaluate the surface morphology of nanoparticles. nanoparticles were prepared and dried well to remove the moisture content and images were taken by using Brookhaven instrument. Results appear in Fig. 10 (Zhang *et al*., 2016).

(j) *In vitro* drug release study

Drug release tests in vitro were carried out using a USP class II dissolving apparatus (Paddle) with a 75-rpm rotation speed. The prepared nanoparticles powder was placed in size 2 capsule and 900 ml of phosphate buffer (pH 7.4) solution taken in basket and temperature maintained at 37±0.5^{0}c. sample measuring 5 ml were withdrawn at a different time interval at 5min, 15min, 30min, 45min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, and 8hr and same volume was replaced with phosphate buffer pH 7.4 solution to maintain sink condition. The sample were examined using a UV- Visible spectrophotometer at 220nm. A % CDR was determined Fig. 11 and Table 12 contain the results (Supriya *et al.*, 2018).

(k) Kinetic study of *In vitro* drug release

The release data result shown in Table 13.

Zero-Order Kinetics:

Zero-order release kinetics describe systems where the drug release rate is constant over a period of time.

$$\mathbf{Qt=Q_0 + K_0t}$$

where Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0 = 0) and K0 is the zero-order release constant expressed in units of concentration, and it is the time at which the drug release is calculated or measured.

First order:

Thiskineticsdescribesconcentrationdependentdrugreleasefromtheformulation.

$$\log C = \log C0 \text{ ñ} Kt/2.303$$

where, C0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time.

Higuchi Model:

This model helps to study the release mechanism of water-soluble and less water-soluble drugs in corporated insemi-solid and solid matrixes**.** Higuchi equation simplified form,

$$Qt = KH.t1/2$$

Where, Qt is the amount of drug released in time t, KH is the Higuchi dissolution constant

Hixon-Crowell Model:

The Hixson-Crowell cube root law describes the release from system where there is a change in surface area and diameter of particles or tables hence ,particles of regular area are proportional to the cube root of its volume.

$$W01/3\text{-}Wt/3=Ks$$

Where, W0 is the initial amount of drug in the pharmaceutical dosage, Wt is the remaining amount of drug in the pharmaceutical dosage form at time t, Ks is the Hixson Crowell constant describing surface volume relation.

Korsmeyer-Peppa's model

To verify the fact that whether the diffusion follows Fick's lawor not, the drug release data can also be plotted against Log time according to Peppa's equation. The drug release can be expressed as,

$$Q = K\ t\ n$$

Where, Q is the cumulative % drug release, t is the time (Mohideen *et al.*, 2013).

(l) *In- vitro* anti-diabetic study

α- Amylase Inhibition Assays

A mixture of 1 ml of starch solution and 1 ml of drugs with concentrations ranging from 2.5 to 160 g/ml was used. This was given 1ml of the enzyme solution and allowed to react at 25°C for 3 minutes. A solution of the colorimetric colour reagent was then added in 1 ml. On a boiling water bath, the ingredients were heated for 10 to 15 minutes. The reduction of 3,5-nitro salicylic acid to 3-amino-5-nitro salicylic acid was used to measure the production of maltose. At 530 nm, the reaction, which caused the colour to change from orange to red, was quantified in comparison to the reagent blank. The usual treatment was acarbose. % Inhibition determined by using formula:

Inhibition activity (%)

$$= \frac{\text{Abs (control) - Abs (extract)}}{\text{Abs (control)}} \times 100$$

Acarbose is an antidiabetic drug used to treat type 2 diabetes mellitus. It is used as positive control. Results shown in Table 14 and 15 and in Fig. 12 (Preethi *et al*., 2018).

RESULT AND DISCUSSION

1. Pre-Formulation Study of Drug:

(a) Organoleptic Properties:

The organoleptic characteristics of drug sample were examined, and they show in Fig. 1 and Table 2.

Fig. 1. *Quassia amara* L.

In pre-formulation study, the identification of sample was performed and results of organoleptic properties of drug was found to be similar to that mentioned in official monograph.

Table 2: Organoleptic Properties of *Quassia amara* L.

Identification Test	Observed Result	Reported Standard
Color	Yellowish	Yellowish
Odor	Bitter	Bitter
Appearance	smooth	smooth

(B) Melting Point: The capillary tube method used to calculate the melting point. The results appear in a table 3.

Table 3: Melting Point of *Quassia amara* L.

Drug	Reported M.P.	Observed M.P.
Quassia amara L.	208°C	203C- 206°C

The melting point of drug was found to be 203°C to 206°C which is very near to reported standard i.e., 208°C. hence we conclude that *Quassia amara* L. was pure in quality.

(C) Solubility Study: The solubility of *Quassia amara L.* was checked in different solvent like water, Acetonitrile, DMSO. Results of solubility are mentioned in Table 4.

Table 4: Solubility of *Quassia amara* L.

Solvent	Solubility (mg/ml)	Inference
Water	-	Insoluble
Acetonitrile	2.1 mg/ml	Soluble
DMSO	1.7 mg/ml	Soluble

Drug is insoluble in water and it shows highest solubility in acetonitrile as compared to DMSO.

(d) UV- Visible Spectroscopy of *Quassia amara* L.

The UV- Spectrum of *Quassia amara* L. extract solution in phosphate buffer at pH 7.4 exhibits a wavelength of absorbance maximum of 220nm. The spectrum of *Quassia amara* L. appear in Fig. 2.

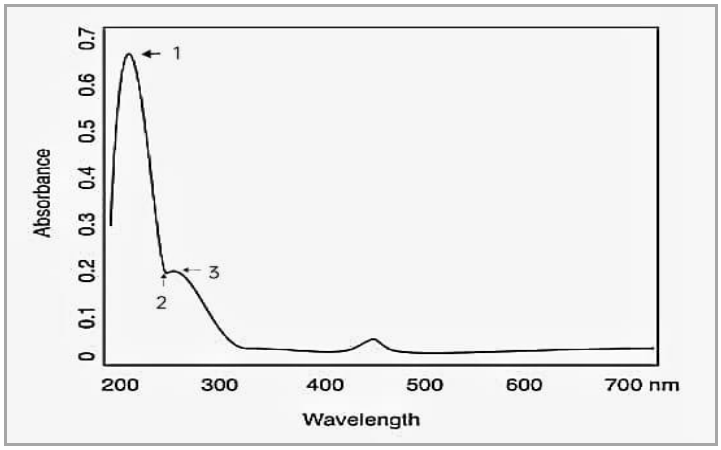

Fig. 2. spectrum of *Quassia amara* L. in Phosphate buffer pH 7.4.

Construction of Beer lamberts plot in Phosphate buffer pH 7.4 :

The calibration curve was found to be linear in the concentration range of 4-20 µg/ml, with a coefficient of regression of R2 = 0.9906 and a slope y = 0.0039x + 0.0042 in Phosphate buffer pH 7.4. result in Table 5 and Fig. 3.

(e) Fourier- transform infrared spectroscopic studies (FT-IR)

The FT-IR spectrum of *Quassia amara* L. was recorded using FT-IR spectrophotometer. The results appear in a Fig. 4 and Table 6.

Table 5: Absorbance of *Quassia amara* L. extract in Phosphate buffer pH 7.4.

Sr. No.	Concentration(µg/ml)	Absorbance
1.	0	0.000
2.	4	0.022
3.	8	0.039
4.	12	0.052
5.	16	0.065
6.	20	0.081

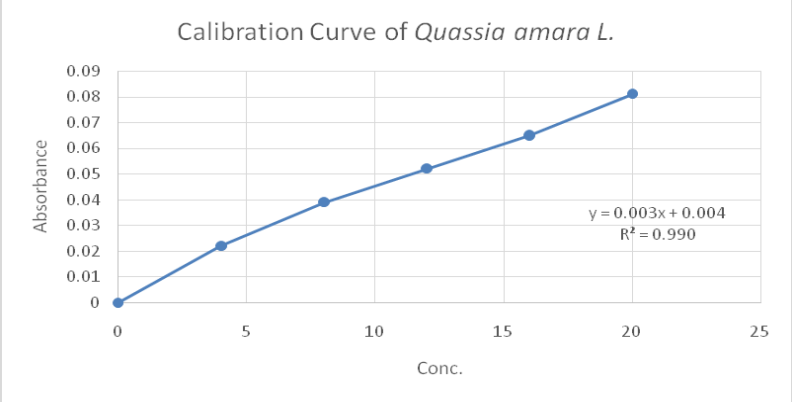

Fig. 3. Calibration curve of *Quassia amara* L. in Phosphate buffer pH 7.4.

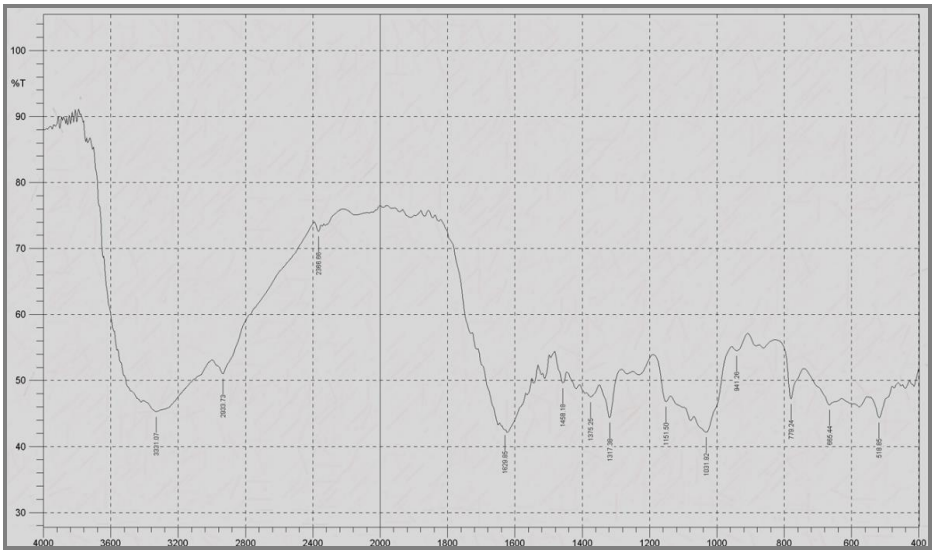

Fig. 4. FTIR spectra of *Quassia amara* L**.**

Table 6: IR-Peaks of *Quassia amara* L.

Material	Standard wave number range	Test wave number	Inference
***Quassia amara* L. bark**	3300 – 3100	3331.07	C-H stretching aromatic ring
	3000 – 3100	2933.73	C-H stretching aromatic ring
	2400 – 2100	2366.66	C-O bond stretching
	1870 – 1500	1629.85	C=O carbonyl stretching in amide
	1390 – 1310	1317.38	Alkanes
	690 – 900	779.24	Alkenes

The FTIR spectra shows that *Quassia amara* L. is pure in nature.

(f) Fourier- transform infrared spectroscopic studies (FT-IR)

The FT-IR spectrum of silver nitrate was recorded using FT-IR spectrophotometer. The results appear in a Fig. 5 and Table 7.

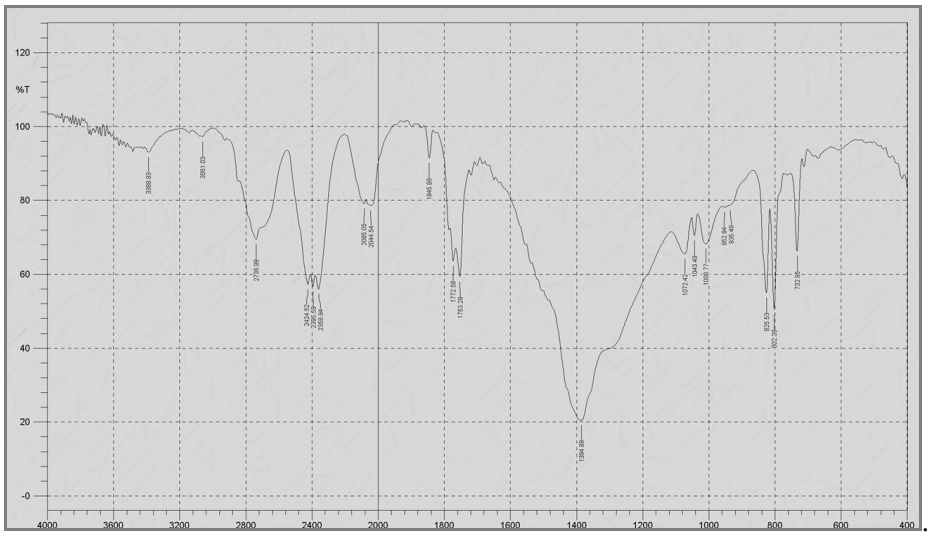

Fig. 5. FTIR spectra of silver nitrate.

Table 7: IR-Peaks of Silver Nitrate.

Material	Standard wave number range	Test wave number	Inference
Silver Nitrate	3550-3200	3388.93	O-H Alcohol
	3200 - 2700	3061.03	O-H Alcohol
	3200-2700	2736.99	O-H Alcohol
	1550-1300	1384.89	N-ONitrocompound

2. Evaluation of Nanoparticles

(a) Visual Examination of Nanoparticles:

The prepared nanoparticles were evaluated for colour, odour and texture. The results appear in a Fig. 6 and Table 8.

Fig. 6. Formulated *Quassia amara* L. Nanoparticles.

Table 8: Physical parameter of nanoparticle.

Identification Test	Observed Result
Color	Brown
Odor	Bitter
Texture	Granular

The prepared nanoparticles were brown in colour with bitter odour and with granular texture.

(b) Percentage yield of Nanoparticles

The percentage yield of nanoparticles was calculated and results appear in Table 9.

Table 9: Percentage yield of Nanoparticles.

Sr. No.	Formulation	% yield
1.	F1	76.32%
2.	F2	81.12%
3.	F3	65.10%
4.	F4	45.55%

The percentage yield of nanoparticles of Optimised formulation (F2) was found to be 81.12%.

(c) Drug Entrapment Efficiency

The % drug entrapment efficiency was appeared in Table 10.

Table 10: Drug Entrapment Efficiency of Nanoparticles.

Sr. No.	Formulation	Drug Entrapment Efficiency
1.	F1	71.5%
2.	F2	92.5%
3.	F3	56.4%
4.	F4	53.7%

Drug entrapment efficiency ranges from 71.5% to 53.7%, which indicate that the amount of drug present in nanoparticle. The drug entrapment of optimized formulation (F2) was found to be 92.5%.

(d) Drug Content

The concentration of the drug was computed from the standard curve of *Quassia amara* L. nanoparticles. The drug content the results appear in a Table 11.

Table 11: Drug Content of *Quassia amara* L.

Sr. No.	Formulation	% Drug Content
1	F1	72.4%
2	F2	95.6%
3	F3	75.7%
4	F4	65.9%

The percentage drug content ranges from72.7% to 65.9%.

(e) FTIR Spectroscopy of Formulation (F2)

In the *Quassia amara* L. bark and wood FTIR spectrum strong absorption peaks at 3331.07 and 2933.73 indicates C-H stretching due to the presence of aromatic ring. Peaks at 2366.66 indicates C-O stretching bond and peaks at 1629.85 indicates C=O stretching due to the presence of amide and N-O stretching of nitro compounds absorption peaks at 1317.38, and 779.24 indicates the presence of alkanes, and aromatic rings. The FT-IR spectrum of formulation was recorded using FT-IR spectrophotometer. Results appear in Fig. 7 and Table 12.

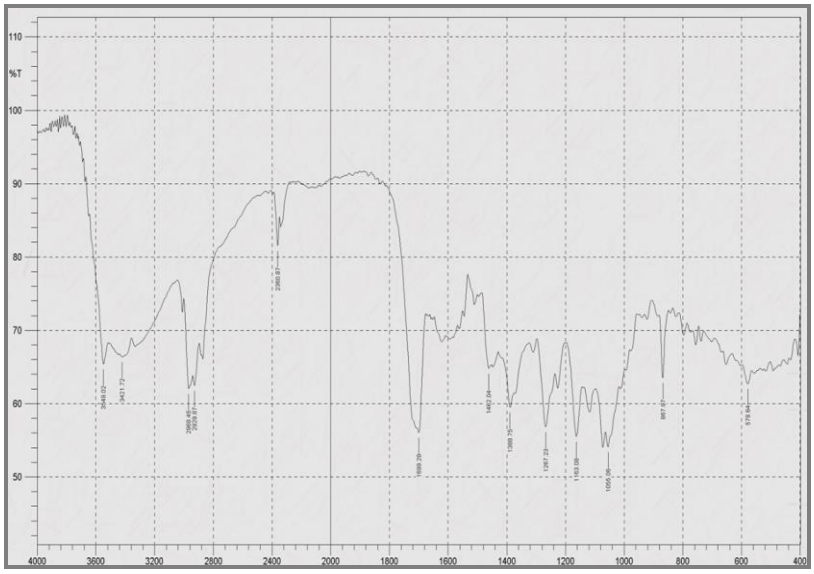

Fig. 7. FT-IR spectra of Optimized batch(F2).

Table 12: FTIR spectra of Optimized batch(F2).

Material	Standard wave Number Range	Test wave number	Inference
***Quassia amara* L. Nanoparticles**	3300 – 3100	3549.02	OH stretching
	2400 – 2100	2366.66	C-O bond stretching
	3000-2900	2968.45	CH stretching in aldehyde
	1870 – 1500	1629.85	C=O carbonyl stretching in amide

The peak corresponds to 1384 cm^{-1} of N-O stretching to be missing in FTIR spectra of F2 formulation. It was found with reduced intensity in FTIR spectra of F2 formulation. This indicate that drug in completely being encapsulated within FTIR.

(f) Particle size Measurement

The mean particle size analysis was done with the help of zeta sizer (Malvern). The outcome shown in Fig. 8.

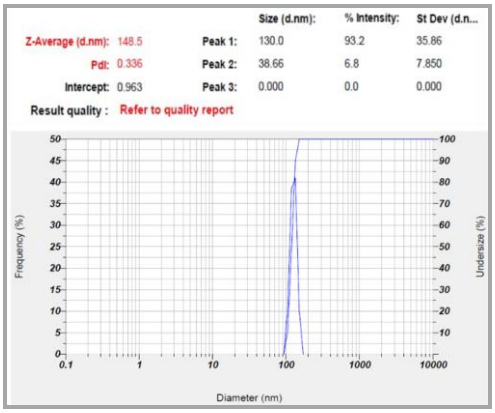

Fig. 8. Particle Size of *Quassia amara* L. Nanoparticle.

The particle size of nanoparticle by using solvent evaporation 148.5nm.

(g) Poly-dispersity Index

The of polydispersity index according to its type of dispersion shown in fig 8.

The poly dispersibility index of optimized formulation (F2) was found to be 0.336.

(h) Zeta Potential Measurement

The zeta potential of nanoparticle is commonly used to characterized the surface charged property of nanoparticles. The results appear in Fig. 9.

The zeta potential of nanoparticle of Optimized batch (F2) was found to be -28mV.

(i) Scanning Electron Microscopy (SEM)

SEM is used to characterise the morphology and particle structure of nanoparticles, with the results depicted in Fig. 10.

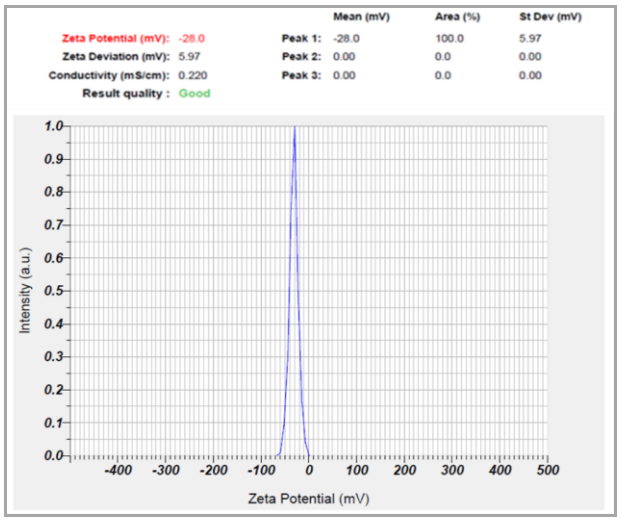

Fig. 9. Zeta Potential of *Quassia amara* L. Nanoparticles.

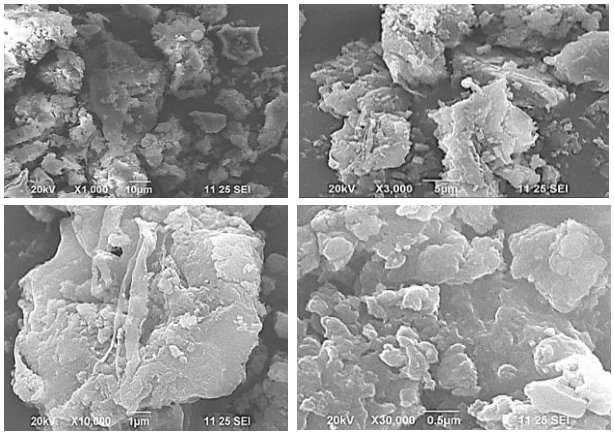

Fig. 10. Scanning Electron Microscopy (SEM).

The magnification images shows that the polydisperse spherical shaped nanoparticles and highly distributed with aggregation.

(j) *In-vitro* drug release study

The cumulative percentage drug release was calculated. The results appear in a Table 13 and Fig. 11.

Table 13: In-vitro drug release study of *Quassia amara* L Nanoparticles.

Time	Cumulative % Drug Release			
	F1	F2	F3	F4
5	31.53%	31.53%	25.05%	25.05%
15	33.81%	36.50%	31.50%	32.05%
30	37.79%	41.52%	34.71%	32.48%
45	39.48%	45.05%	37.40%	32.90%
60	44.17%	49.38%	39.17%	33.33%
120	47.25%	52.99%	42.17%	33.91%
180	50.03%	56.63%	44.07%	34.35%
240	54.04%	61.07%	47.60%	34.94%
300	59.83%	64.79%	50.98%	35.68%
360	63.62%	68.54%	52.91%	36.20%
420	66.42%	71.56%	55.92%	36.80%
480	76.99%	82.29%	59.78%	37.01%

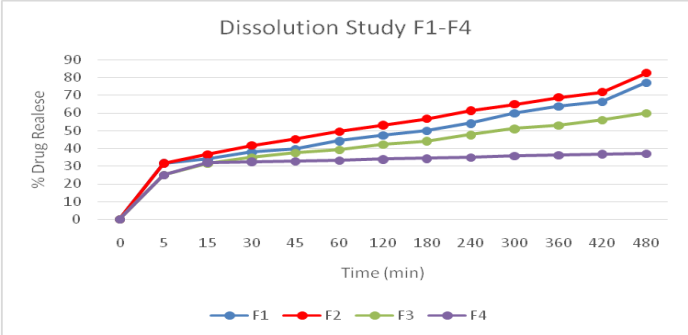

Fig. 11. Dissolution Study of F1 To F4.

The *in vitro* dissolution showed that F2 batch release *Quassia amara* L. up to 82.29% in 8hrs (480min). Hence F2 optimized formulation and kinetic treatment was applied for the same.

(k) Kinetic Assessment of Formulated Nanoparticles of *Quassia amara* L.

Table 14: Regression coefficient value of *Invitro* studies.

Batches	Coefficient of Regression R^2					
	Zero order	First order	Higuchi model	Hixon model	Korsmeyer Peppas	
					R^2	n
F1	0.9029	0.8392	0.9752	0.8464	0.9823	2.9
F2	**0.9421**	0.9336	**0.9731**	0.9469	0.9143	**19.9**
F3	0.6007	0.6947	0.6939	0.6999	0.6915	1.8
F4	0.6006	0.9644	0.7197	0.9646	0.8420	0.8

The kinetic data applied on in-vitro dissolution studies shows that optimised batch F2 follows zero order kinetics and Higuchi model. Which shows that drug release by diffusion mechanism. The n exponent value of F2 batch was found to be 19.9. this value was greater than 0.9 value, which means that F2 batch follows super case II transport drug release mechanism.

(l) *In- vitro* Antidiabetic study

In-vitro antidiabetic study exhibits good alpha amylase inhibition under *in-vitro* condition. The results of alpha amylase inhibitory effect of positive control acarbose shown in table 15 and the results of alpha amylase inhibitory effect of *Quassia amara* L. nanoparticles shown in Table 16. And the comparison of alpha amylase inhibition of Acarbose vs *Quassia amara* L. nanoparticles were shown in Fig. 15.

Table 14: Alpha amylase inhibitory effect of Positive control Acarbose.

Concentration (mg/ml)	Absorbance	% Inhibition
0.2	0.825	33.69%
0.4	0.755	39.31%
0.6	0.619	50.24%
0.8	0.531	57.32%
1.0	0.330	73.47%

Table 15: Alpha amylase inhibitory effect of *Quassia amara* L. Nanoparticles.

Concentration (mg/ml)	Absorbance	% Inhibition
0.2	0.81	34.89%
0.4	0.779	37.38%
0.6	0.714	42.61%
0.8	0.691	44.46%
1.0	0.455	63.43%

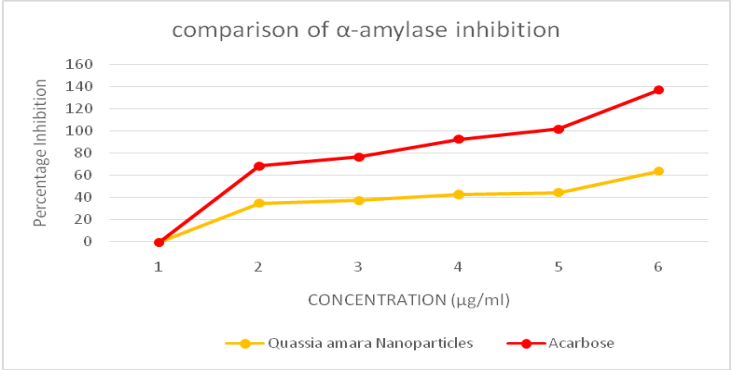

Fig. 12. α-amylase inhibition of *Quassia amara L* nanoparticles vs Acarbose.

The percentage α amylase inhibition of positive control Acarbose at lower and higher concentration was found to be 33.69% and 73.47%. and the percentage α-amylase inhibition at lowest and highest concentration were found to be 34.89% and 63.43% respectively.

DISCUSSION

Formulating nanoparticles of *Quassia amara* L. enhances its therapeutic potential by improving bioavailability, stability, and targeted drug delivery. The controlled release and reduced dosage requirements contribute to its efficacy while minimizing adverse effects, making it a promising option for the treatment of various diseases, including diabetes. Solubility of *Quassia amara* L. in water (hydrophilic), acetonitrile (partially soluble), and DMSO (lipophilic) observed (Mehnert and Mäder 2001). Implications for formulation and therapeutic applications considered. The UV spectrum of *Quassia amara* L. extract in pH 7.4 phosphate buffer showed an absorbance maximum at 220nm, (Pawar *et al*., 2022) indicating the presence of specific chromophores and potential pharmaceutical applications and regression of R^2 is 0.9906. FTIR spectra of *Quassia amara* L. bark and wood revealed significant peaks at 3331.07 cm^{-1} and 2933.73 cm^{-1} (C-H stretching), 2366.66 cm^{-1} (C-O stretching), and 1629.85 cm^{-1} (C=O stretching) indicating aromatic rings, amides, and nitro compounds. Peaks at 1317.38 cm^{-1} and 779.24 cm^{-1} suggest alkanes and aromatic rings. (Holler, sixth edition) These spectral features provide valuable insights into the chemical composition and potential pharmaceutical applications of *Quassia amara* L. The optimized formulation (F2) demonstrated a high percentage yield of nanoparticles at 81.12%, indicating an efficient and reproducible nanoparticle synthesis method. The drug entrapment efficiency of nanoparticles varied between 71.5% to 53.7%, signifying drug content in the nanoparticles. For optimized formulation (F2), drug entrapment was 92.5%, indicating effective drug encapsulation. Drug content in nanoparticles varies from 72.7% to 65.9%, demonstrating consistent drug encapsulation across formulations. Nanoparticles prepared via solvent evaporation exhibited a mean particle size of 148.5 nm, accompanied by a low polydispersity index (PDI = 0.336) and a zeta potential of -28mV (Supriya *et al.*, 2018). Microscopic analysis confirmed polydisperse, spherical-shaped nanoparticles with widespread distribution and some instances of aggregation (Xi-Feng Zhang *et al.*, 2016). The F2 batch of *Quassia amara* L. nanoparticles demonstrated 82.29% release in 8hrs (480min) in vitro dissolution (Supriya *et al.*, 2018). The optimized formulation and kinetic analysis were employed for further evaluation. In-vitro dissolution studies revealed that optimized batch F2 adheres to zero-order kinetics and the Higuchi model. (Mohideen *et al*., 2013) Positive control Acarbose exhibited dose-dependent α-amylase inhibition (33.69%-73.47%), while test compound demonstrated inhibition in the range of 34.89%-63.43% (Preethi *et al*., 2018).

CONCLUSIONS

The developed formulation exhibits good organoleptic qualities, according to all the observations and results. When both drug and excipient were characterised, no undetectable peaks were found in the FT-IR study. The entrapment efficiency was found to be optimised formulation is 92.5%. the drug content of prepared nanoparticles ranges between 71.5% to 53.7%. Th particle size of then a no particles is 148.5 nm. The zeta potential was found to be -28 mV, which indicates good formulation stability. One of the main benefits of nanoparticles over conventional drug administration is this. The *in-vitro* drug release study confirmed that *Quassia amara* L. was released for a long time. The kinetic assessment of in-vitro release of *Quassia amara* L. nanoparticles was also performed. Finally, we derived from this comprehensive analysis that site-specific activity can be successfully achieved using *Quassia amara* L. nanoparticle formulation.

FUTURE SCOPE

To perform toxicity.
Biomedical applications they can be utilized for targeted drug delivery.
To perform in-vivo studies.
To perform ex-vivo studies.

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