DEVELOPMENT AND EVALUATIONOF ANTI DIABETIC ACTIVITY OF *Cassia auriculata* Linn NANOPARTICLES

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MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND EVALUATION OF ANTI DIABETIC ACTIVITY OF *Cassia auriculata* Linn NANOPARTICLES**" submitted by **Mrs. ANNAL THAMARAISELVI.J (Reg .No: 261710101)** in partial fulfillment for the award of **Master of Pharmacy** in Pharmaceutics under The **Tamilnadu Dr.M.G.R Medical University**, Chennai, done at K.M. COLLEGE OF PHARMACY, Madurai-625107, is a bonafide work carried out by her my guidance and supervision during the academic year 2018-2019. The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universitity.

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EXPRESION OF GRATITUDE

“With God All Things Are Possible

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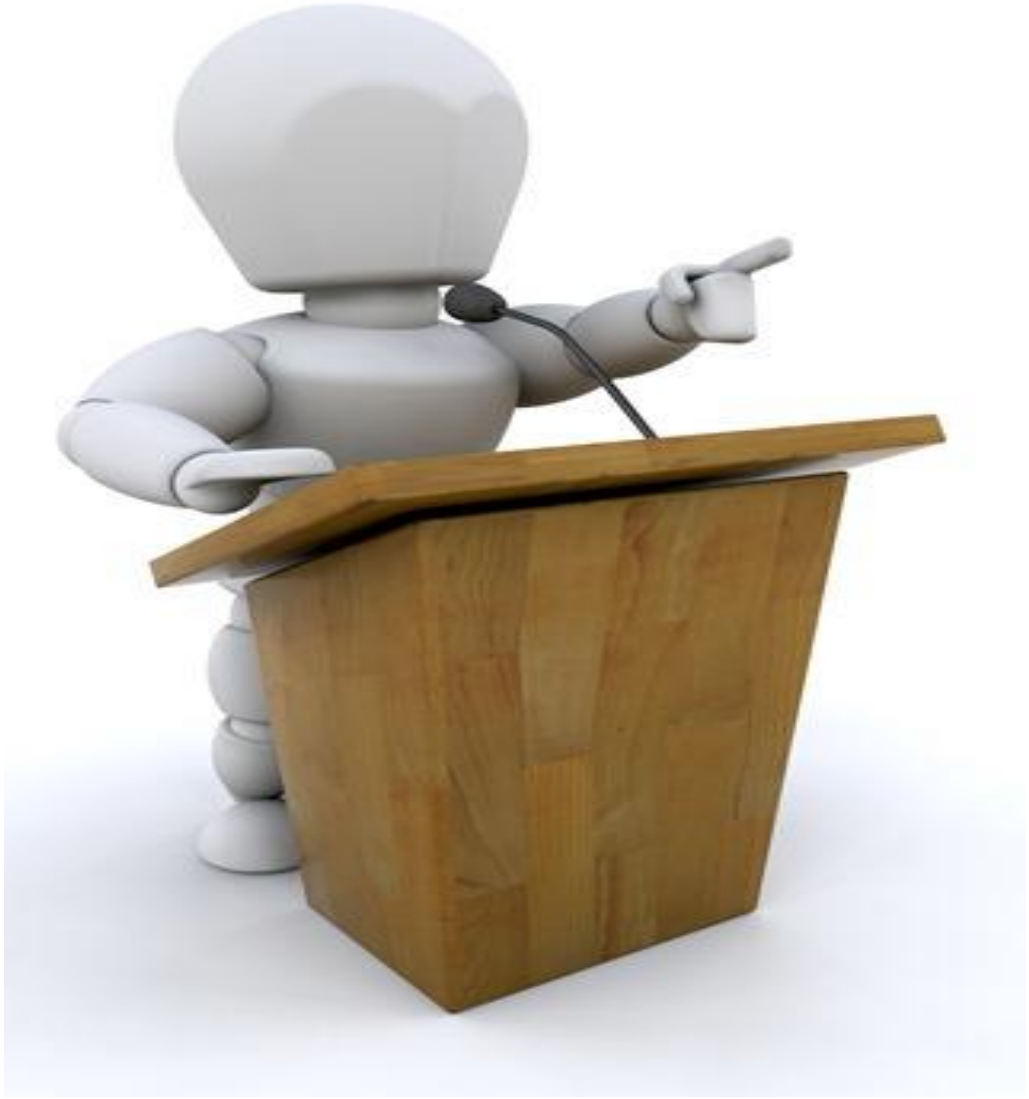
DEDICATED TO ALMIGHTY

OUR BELOVED PARENTS

& TEACHERS

AND

MY BROTHERS AND FRIENS



INTRODUCTION

REVIEW OF LITERATURE

RESEARCH INVISAGED

MATERIALS AND METHODS

DRUG PROFILE

EXPERIMENTAL INVESTIGATION

RESULTS AND DISCUSSION

CONCLUSION

BIBLIOGRAPHY

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INTRODUCTION

1.1 Targeted drug delivery system (TDDS)[1]

It is a smart drug delivery system of delivering medication to a patient in a manner that increases the concentration of the medication to part of the body relative to others. This means of delivery is largely founded on nanomedicine, which plans to employ nanoparticle-mediated drug delivery in order to combat the downfalls of conventional drug delivery.

These nanoparticle would be loaded with drugs and targeted to specific parts of the body where there is solely diseased tissue, thereby avoiding interaction with healthy tissue. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue.The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system releases the drug in a dosage form.

The advantages to the targeted release system is the reduction in the frequency of the dosages taken by the patient, having a more uniform effect of the drug, reduction of drug side-effects, and reduced fluctuation in circulating drug levels.The disadvantage of the system is high cost, which makes productivity more difficult and the reduced ability to adjust the dosages.Targeted drug delivery systems have been developed to optimize regenerative techniques.The system is based on a method that delivers a certain amount of a therapeutic agent for a prolonged period of time to a targeted diseased area within the body

This helps maintain the required plasma and tissue drug levels in the body, thereby preventing any damage to the healthy tissue via the drug. The drug delivery system is highly integrated and requires various disciplines, such as chemists, biologists, and engineers, to join forces to optimize this system.

Advantages of TDDS [2]

- Convenience in Administration,
- Non invasive,
- accurate dose,

Disadvantages of TDDS

- Unconscious patients cannot take dose,
- Low solubility and permeability,
- Degradation by Gastro Intestinal flora,
- first pass metabolism,
- Food interactions,
- Poor bioavailability.

Types of Targeted Drug delivery systems: [3]

Nanoparticle:

Particle size ranges from 10^{-9}m .Microscopic particle size is measured in nano meters. Small object that behaves as a whole unit with respect to its transport and properties.

Nanocrystal:

Smaller than 100 nanometres. Composed of atoms in either a single- or poly-crystalline arrangement.

Nanowires:

Wire with a diameter of only a few nanometres. Structures that have a thickness or diameter constrained to Tens of **nanometers** or less and an unconstrained length

Nanotubes:

Tubular molecule consists of a large number of carbon atoms.

Micro emulsion:

Clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, Aqueous phase contain salt other ingredients oil phase contain complex mixture of different hydrocarbons and olefins.

Applications of TDDS: [4]

Targeted drug delivery used to treat various diseases like

- cardiovascular diseases,
- Cancer tumors

- diabetes,
- tuberculosis,
- CNS,
- Oncology
- Cardiovascular,
- pulmonary
- infectious disease

1.2 Nanoparticles [5]

Nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit. Size-Related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually Not referred to as nanoparticles. Nanoclusters have at least one dimension between 1 and 10 Nanometers and a narrow size distribution Nanopowdersare agglomerates of ultrafine Particles, nanoparticles, or Nanoclusters. Nanometer-sized single crystals, or single-domain ultrafine Particles are often referred to as Nanocrystal. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. The National Nanotechnology Initiative has led to generous public funding for nanoparticle research in the United States.

Advantages of Nanoparticles:

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration
- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug
- Therapeutic efficacy and reduction in side effects.

Methods of targeting [6]

Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of

- Magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc Nanoparticles can better deliver drugs to tiny areas within the body.
- Engineering on this scale enables researchers to exercise exquisite and previously unthinkable control over
- The physical Attributes of polymers and other biomaterials.
  Nanoparticles overcome the resistance offeredby the physiological barriers in the body because efficient delivery of drug to various parts of the body is directly affected by particle size.

- Nanoparticles aid in efficient drug delivery to improve aqueous solubility of poorly soluble
- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.
- Polymers used in preparation of nanoparticles
- The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and
- (Non-antigen city)and should be biodegradable and biocompatible

POLYMERS [7-12]

Natural polymers:

The most commonly used natural polymers in preparation of polymeric nanoparticles are 17-20.

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

Synthetic polymers

- Polylactides(PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters
- Polycyanoacrylates
- Polycaprolactone
- Poly glutamic acid
- Poly malic acid
- Poly(N-vinyl pyrrolidone)
- Poly(methyl me 13thacrylate)
- Poly(vinyl alcohol)
- Poly(acrylic acid)
- Poly acrylamide
- Poly(ethylene glycol)
- Poly(methacrylic acid)

1.3 Preparation Nanoparticles [13]

Methods for preparation of nanoparticles from dispersion of preformed polymer Dispersion of drug in preformed polymers is a common technique used to prepare biodegradable

Nanoparticles from poly (lactic acid) (PLA), poly (D, L-glycolide) (PLG), poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA). These can be accomplished by different methods described below.

- Solvent evaporation
- Spontaneous emulsification or solvent diffusion method
- Polymerization method
- Polymerization method
- Coacervation or ionic gelation method

Solvent evaporation method [14]

- In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug.
- The mixture of polymer and Drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion.
- After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring.
- Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration.
- In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

Spontaneous emulsification or solvent diffusion method [15]

- This is a modified version of solventevaporation method. In this method, the water insoluble solvent alongWith a small amount of thewater immiscible organic solvent is used as an oil phase.

- Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved.
- Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with thedrug dissolved in the internal aqueous phase.

Polymerization method [16]

- In this method, monomers are polymerized to form nanoparticle in an aqueous solution. Drugs incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed.
- The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium.
- This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles.

Coacervation or ionic gelation method [17]

- The nanoparticles preparation is carried by using biodegradable hydrophilic polymers such as chitosan,Gelatin and sodium alginate.
- Developing a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. In this method, positively charged amino-group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanoparticles.

1.4 Evaluvation of Nanoparticle [18]

Particle Shape

SEM characterizes the nanosuspension before going for evaluation; the nanosuspension is lyophilized toform solid particles. The solid particles are coated with platinum alloy using a sputter coater.

Particle size

Particle size and size distribution are the most important characteristics of nanoparticle systemstheydetermine the in vivo distribution, biological fate, and toxicity and targeting ability of nanoparticle system. In addition, they can also influence the drug loading, drug release and stability of nanoparticle

Particle Size and Zeta Potential

Value of Particle size and Zeta Potential prepared nanoparticles determined by using Malvern Zetasizer

Zeta potential

The Zeta potential of a nanoparticle is commonly used to characterized the surface charge Property of nanoparticles.It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticle with a zeta potential above (±) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles.

Surface Morphology

Surface morphology study carried out by Scanning Electron Microscopy (SEM) of prepared nanoparticle

Polydispersity index

Polydispersity index of prepared nanoparticles was carried out by using Malvern Zetasizer

Size determination

A laser light scattering particle size analyzer was used to determine the particle size of the nanoparticle formulation Samples were suspended in distilled water and stirred continuously during the particle size analysis. The size distribution was expressed by the volume median diameter (VMD) and span value Span is a measure of the width of the size distribution. Where D (v,90), D (v,10) and D (v,50) are the equivalent volume diameters at 90%, 10% and 50% cumulative volume

Stability of Nanoparticles [19]

Stability studies of prepared nanoparticles determined by storing optimized formulation at 4°C ±1°C and 30°C ± 2°C in stability chamber for 90 days. The samples were analyzed after a time period like at 0, 1, 2And 3 months for their drug content, drug release rate (t50%) as well as any changes in their physical appearance

Drug Entrapment Efficiency [20]

The nanoparticles were separated from the aqueous medium by ultracentrifugation at 10,000 rpm for 30 min at 50C. Then the resulting supernatant solution was decanted and dispersed into phosphate buffer saline pH 7.4. Thus the procedure was repeated twice to remove the entrapped drug molecules completely.The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

Drug Entrapment efficiency (%) = Amount of released from the lyses nanoparticle X 100 Amount of drug Initially taken to prepare the nanoparticles The process of SAS employs a liquid solvent, eg methanol which is completely miscible with the supercritical fluid (SC CO2) to dissolve the solute to be micronized at the process conditions, because the solute is insoluble in the supercritical luid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles.

RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region Lower pressure 21, thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles supercritical Fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.

Limitations of Nanoparticles [21]

Small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

1.5 Disease of Profile [22]

Diabetes Mellitus symptoms of Include

- increased thirst and urination
- increased hunger
- fatigue
- blurred vision
- numbness or tingling in the feet or hands
- sores that do not heal
- unexplained weight loss

Symptoms of type 1 diabetes can start quickly, in a matter of weeks. Symptoms of type 2 diabetes often develop slowly—over the course of several years—and can be so mild that you might not even notice them. Many people with type 2 diabetes have no symptoms. Some people do not find out they have the disease until they have diabetes-related health problems, such as blurred vision

Type 1 diabetes:

Type 1occurs when your immune system, the body's system for fighting infection, attacks and destroys the insulin--producing beta cells of the pancreas. Scientists think type 1 diabetes is caused gene environmental factors, such as viruses, that might trigger the disease. Studies such as Trial Net are working to pinpoint causes of type 1 diabetes and possible ways to prevent or slow the disease.

Type 2 diabetes

Type 2 diabetics the most common form of diabetes is caused by several factors, including lifestyle factors and genes.

Overweight, obesity, and physical inactivity

Type 2 diabetes if you are not physically active and are over weight and obese weight sometimes causes insulin resistance and is common in people with type 2 diabetes. The location of body fat also makes a difference. Extra belly fat is linked to insulin resistance, type 2 diabetes, aheart and blood vessel disease. To see if your weight puts you at risk for type 2 diabetes, check out these Body mass indexes (BMI) Charts.

Insulin resistance

Type 2 diabetes usually begins with insulin resistance, a condition in which muscle, liver, and fat cells do not use insulin well. As a result, your body needs more insulin to help glucose enter cells. At first, the pancreas makes more insulin to keep up with the added demand. Over time, the pancreas can’t make enough insulin, and blood glucose levels rise.

Genes and family history

As in type 1 diabetes, certain genes may make you more likely to develop type 2 diabetes. The disease tends to run in families and occurs more often in these racial Genes also can increase the risk of type 2 diabetes by increasing a person’s tendency to become overweight or obese.

1.6 PLANT PROFILE

Cassia auriculata.L used for long period in various chronic diseases therapeutically. Aim of the current review is to search literature for the pharmacological properties, safety/ toxicity studies, pharmacognostic studies and phytochemical investigation of *Cassia auriculata*.L plant. Particulars of pharmacological activities, phytochemical isolation, toxicity studies etc. were extracted from the published reports focusing on the safety profile of the plant. Safety of the whole plant was concluded in the review. The compiled data may be helpful for the researchers to focus on the priority areas of research yet to be discovered

Fig. 1 Pant of Cassia auriculata .L [42]

Plant description:

Cassia auriculata Linn commonly known as Tanners Senna, is also known as Avaram tree.

Distribution:

It is distributed throughout hot deciduous forests of India. Wild in dry regions of Madhya Pradesh, Tamil Nadu, Rajasthan and other parts of India.

Fig.2. Flower of *Cassia auriculata*.L Flower

Flowers:

- Irregular, bisexual, bright yellow and large (nearly 5 cm across)
- The pedicels glabrous and 2.5 cm long.
- The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes).
- The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones.
- The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange.
- The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules.

- *Cassia auriculata.*L

Botanical Name : *Cassia auriculata.*L

Common Name : Tarwar

Morphological characters: [23]

- *Cassia auriculata*.L is a branched shrub.
- Growing up to 1-1.5 m high.
- It has a smooth reddish brown bark.
- It has many ascending branches and 8-10 cm long pinnate leaves.
- There are 8-12 pairs of leaflets, each 2-3 cm long.
- Bright yellow flowers appear in racemes at the end of branches.
- The flowers are 4-5 cm across.
- Upper three stamens are reduced to stamenoides.
- Fruit is a 7-12 cm long, flat brown pod.
- Flower of *Cassia auriculata*.L

Growing Season and Type:

1. *Cassia auriculata*.L is suitable for landscaping roadways and home gardens. It tolerates drought and dry conditions, but not much cold. The flowers in racemes are also attractive.

2. It starts flowering and fruiting at the age of 2-3 years. Once established, it flowers precociously and abundantly throughout the year.

3. It prefers properly drained soil

Medicinal Use [24]

- The plant has been reported to possess antipyretic
- hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic and microbicidal activity
- *Cassia .auriculata* has been shown to antiviral activity and anti spasmodic activity. The plant is used in the traditional system of medicine for female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam
- The plant has been widely used as a cure for rheumatism and conjunctivitis

- The various parts of the plant were reported to exert a beneficial effect to alleviate the symptoms of diabetes
- The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation

Preparation of Flower extract

- *Cassia auriculata.*L flowers were shade dried and powdered. 500g of powdered material was extracted with 1500 ml of methanol for 72h.
- The solvent was evaporated under reduced pressure using rota-evapoator.
- The final yield of crude extract was used for partial purification

Anti Diabetic Activity of Plant Cassia auriculata.L [109]

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes disturbance in carbohydrate, protein and lipid metabolism and complications such as retinopathy, micro angiopathy and nephropathy. In practical terms, diabetes mellitus is a condition in diabetes, a profound alteration in the concentration and composition of lipid occurs. The global figure of people with diabetes set rise from the current estimate of 150-220 million in 2010 and 300 million in 2025.

Despite the immense strides that have been made in the understanding and management of diabetes the disease and disease related complications are increasing unabated. In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plant are used with success to treat this disease. Many traditional plants treatments for diabetes are used throughout the world and there is an increasing demand by patients to use the natural products with anti-diabetic activity.

The present investigation is undertaken to the study the effect of Ethanolic extract of *Cassia auriculata* and its nano particles preparation on changes in Body weight, Plasma glucose, Hemoglobin and glycosylated hemoglobin and lipid profile.

K R C Reddy,*et al*., (2015) reported *Cassia auriculata* used for long period in various chronic diseases therapeutically. The current review is to search literature for the pharmacological properties, safety/ toxicity studies, pharmacognostic studies and phytochemical investigation of *Cassia auriculata*plant. Particulars of pharmacological activities, phytochemical isolation, toxicity studies etc. were extracted from the published reports focusing on the safety profile of the plant. Safety of the whole plant was concluded in the review. The compiled data may be helpful for the researchers to focus on the priority areas of research yet to be discovered.[25]

Ponnusamy,*et al*.,(2014) determined and characterize the chemical constituents of *Cassia auriculata* flower extract by qualitative, quantitative and analytical techniques. Preliminary Phytochemical, total flavonoid and phenol content was determined in the methanolic extract of *C.auriculata* (CAFMEt) using standard methods. C-18 silica gel based column chromatography was used to purify CAFMEt using n-hexane, ethyl acetate and methanol and fraction identified by thin layer chromatography. GC-MS and FT-IR techniques used to characterize the lead fraction. CAFMEt showed the presence of flavonoid and Phenols in a significant amount. Three fractions was collected from column chromatography *viz*., Fraction 1-3 (n-hexane: yellow) was 2.5mg, (ethyl acetate: light orange) 1.8mg and (methanol: light green) 5.67mg respectively. TLC indicated n-hexane has higher refractive factors 0.457 at yellow band and ethyl acetate fraction has 0.329 at light orange band. 14 chemical constituents were identified by GC-MS included alkanes, alcohol, esters and hydrocarbons. The major peak showed the presence of 4-(4-methylphenoxy) phenol at 22.53%. Infra red spectra revealed the presence of phenolic groups in hexane fraction.[26]

Rani, *et al.*, (2014) studied to explore the protective effect of *Cassia auriculata.*L. flower extract (CAE) in high fat diet and streptozotocin induced type 2 diabetic (T2DM) rats.T2DM was induced by a combination of high fat diet and low dose streptozocin. Rats in different groups were treated with *Cassia auriculata.* L. Flower extract at two different doses viz. 300mgand 500mg/Kg body weight and the hypoglycemic potential as wellas lipid lowering and antioxidant properties of CAE in liver and pancreas were evaluated.T2DM rats showed significantly elevated glucose and reduced c-peptide levels in serum. Also there was significant increasein serum marker enzymes of liver

toxicity-alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase(ALP) along with significant reduction in liver glycogen and increase in lipid peroxidation levels. There was also deregulation in lipid levels in plasma and liver and significant reduction in antioxid and enzymes in plasma, liver and pancreas. encouragingly, treatment with *Cassia auriculata. L* extract caused significant improvement in the glucose, insulin, lipid levels in plasma and the antioxidant status ofliver and pancreas.Out of the two doses of CAE used in this study, 500mg/kg b.w dose was found to be more effective in regulating the levels of antioxidants and lipid levels in plasma, liver and pancreas indicating its potential to ameliorate peripheral insulin resistance. [27]

Monisha*, etal*., (2018) investigated *Cassia auriculata.*L used for long period in various chronic diseases therapeutically. Phytochemical studies of leaves and pods of *Cassia auriculata*. Hence it have antimicrobial, antioxidant, anti-inflammatory properties and the present study of aqueous and methanol extracts were used to identify the medicinal properties of *Cassia auriculata.* With reference to the above claims, the results of phytochemical, antioxidant, antimicrobial, anti-inflammatory, chromatography studies of leaves and pods have been described.[28]

Subhadevi, *et al*.,(2014) studied the antibacterial activity of crude and step gradient solvent of methanol, chloroform and benzene in flower and whole plant of Cassia *auriculata.*L and *A. indicum* respectively. The extracts were analyzed for antimicrobial activity using agar well diffusion technique against six bacterial human pathogens viz. *S. typhi, S.flexneriE.coli, V.cholerae, M.tuberculosis, P. fluorescens.* To identify the compound responsible for antibacterial activity the most potent extract was subjected to phytochemical and1HNMR analysis. The growths of *M. tuberculosis and V. cholera* were inhibited by the crude methanol extract of *S. auriculata and A. indicum* respectively. Among the step gradient extract maximum inhibition zone was obtained in Methanol 80%and Benzene 20% and methanol 80% and chloroform 20% in the tested pathogens. Among the tested pathogens *M. tuberculosis, E. coli and V. cholera* were found to be the most susceptible pathogens in both the plants. Phytochemical screening of *S.auriculata* confirmed the presence of alkaloids, flavonoids, triterpenoids and glycosides and in A. indicum alkaloids, tannins, sterols, flavonoids, terpenoids and

saponins. The 1H NMR spectrum of crude methanol extract of *A. indicum* revealed the presence aliphatic methyl (-CH3) protons, aliphatic methylene (-CH2) protons,CH2 X group, alkenic (C-H=C-H) protons and aromatic C-H protons and *S.auriculata* confirmed the presence of CH3/aliphatic methyl group, aliphatic methylene (CH2 alkenic C-H or N-H or (HC=C-H) and aromatic C H protons.[29]

P.Saritha,*et al*., (2017) studied the herbal medicines in developing countries from centuries for safety, efficacy, cultural acceptability and lesser side effects for their primary health care. Due to this increasing trend towards use of therapeutically interesting and important drugscan be developed from plant sources which are used in traditional systems of medicines. *Cassia auriculata.*L commonly known as Tanner's *Cassia* is an important medicinal shrub used in traditional systems of medicine it grows in many parts of India and in other parts of Asia. The flower, leaves, stem, root, and unripe fruit are used for treatment, especially in Ayurveda and Siddha medicine. Aim of the current review is to search literature for the medicinal investigation of cassia auriculata plant. The *Cassia auriculata*(Tangedu) is Telangana state flower, which has enormous traditional uses against various diseases. It is also used for the treatment of pain, fever, urinary tract disorders, rheumatism, conjunctivitis, ulcers and liver disease.[30]

Venkatesh.,*et al*., (*2015)* evaluated an ethanolic extract of flowers of *Cassia Auriculata Linn* was investigated for its analgesic and antipyretic activity. Hot plate method was followed for the investigation of analgesic activity and Yeast induced pyrexia method for antipyretic activity. 200mg/kg and 400mg/kg of ethanolic extract of *Cassia auriculata.*L were used for the study. Aspirin and Paracetamol were used as a standard drug for the investigation of analgesic and antipyretic activity respectively.The study showed significant analgesic and antipyretic activity for the flowers of *Cassia Auriculata*. The results are comparable with that of the standard drug. The study accounts the scientific validation of reported use of the said plant in folklore use [31]

Raja,*et al*., (2013) evaluated the antimicrobial activity of aerial parts of chloroform extract of *Cassia auriculata L*. The chloroform extract of *C. auriculata* were shown to possess an antimicrobial activity against two gram positive and two gram negative human pathogenic bacteria and fungi, *viz*. *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungus cultures *Candida albicans* and

*Aspergillusniger*by using disc diffusion method. The extract showed antibacterial activity at all concentrations selected, but only the extract with the concentration of 300µg/ml showed maximum antibacterial activity against all the organisms except *Pseudomonas aeruginosa* which are comparable with the standard control, amikacin. The anti fungal activity of chloroform extract of *C. auriculata* revealed significant effect against *Candida albicans* and *Aspergillusniger* with the net inhibition zone of 14 and 14 mm, respectively at 300µg/ml concentration, which is almost comparable with standard control, ketokonazole used as an antifungal agent. The phytochemical analysis showed the presence of alkaloids, carbohydrates, fixed oils, fats, tannins, gum & mucilage, flavonoids, saponins, terpenoids, lignin and sterols. The antimicrobial activity showed by the plant was due to the presence of these phytochemicals.[32]

Christian,*et al*.,(2008) reviewed then increased use of engineered nanoparticles and the increased pressure to commercialise this growing technology. Nanoparticles and their preparation and then discuss how these factors can play a role in determining their fate and behaviour in the natural environment. Key focus of the discussion will relate to the surface chemistry of the nanoparticle, which may interact with a range of molecules naturally present in surface waters and sediments. Understanding these factors is a core goal required for understanding the final fate of nano materials and predicting which organisms are likely to be exposed to these materials.[33]

Gupta.,*et al*.,(2011) focused on drug loaded ethyl cellulose (EC) microspheres by oil-in-water (o/w) emulsion solvent diffusion evaporation technique. Aceclofenac (ACF) is an analgesic and anti-inflammatory and diarrhoea, dyspepsia, abdominal pain, nausea, indigestion, pancreatitis, constipation the most common side effects. So the aim of the present research work was to formulation design optimization and investigation of ACF loaded EC microspheres by o/w emulsion solvent diffusion evaporation technique with different ratio of drug and ethyl cellulose as a polymer in order to achieve high entrapment efficiency and prolonged release characteristics. The prepared microspheres were characterized by scanning electron microscopy (SEM), percent yield, micrometrics properties, fourier transformer infra red spectroscopy (FTIR), percent entrapment efficiency and percent drug release characteristics. The size of microspheres formulations (F1 to F6) were in range of 10±2.1 to51±2.7 µm, percent

yield 75.32±2.21 to 95.43± 1.13%, percent drug entrapment efficiency55.87±2.03 to 87.53±2.12% and percent drug release 58.36 ± 0.32 to 94.68 ± 0.54 % up to 12hrs. IR and differential scanning calorimetry (DSC) study showed no interaction between drug and polymer; no degradation during microspheres preparation and stable at storage conditions. All microsphere formulations showed various drug releases kinetic but F2formulation followed first order drug release kinetics and 94.68 ± 0.54 % drug release for prong period of time. From the study, it was investigated that free flowing and spherical microspheres of ACF could be prepared successfully by solvent diffusion evaporation technique with high entrapment efficiency and prolong release profile characteristics.[34]

Maneemegalai.,*et al*.,(2012) Studied antibacterial activity of ethanol, methanol and aqueous extracts of dry flower and ethanol, methanol and acetone extracts of fresh flower of *Cassia auriculata* was conducted using agar disc diffusion method. The micro organisms used include *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiellapneumoniae, Vibrio cholerae*and *Shigelladysentrae.* The maximum activity was observed against all organisms except *Pseudomonas aeruginosa* and *Klebsiellapneumoniae*. The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and various extract. Presence of phytochemicals such as terpenoids, tannins, flavonoids, saponin, cardiac glycosides and steroids were observed. *Cassia auriculata* was observed to have antibacterial activity and can be used for medicinal purposes.[35]

Gayathri,*et al*.,(2018) evaluvated antidiabetic efficiency of bud and flower and to identify the differential composition of phycompounds present in bud and flower parts of *C. auriculata* L. The compounds present in the bud and flower parts were identified using LC-ESI/MS analysis. Antidiabetic activity of *C. auriculata* L. bud and flower parts was studied in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats. During which parameters such as feed intake, water intake, and body weight were monitored. After 21 days of the study, blood parameters like insulin, glucose, lipid profile, hepatic function test, renal function test and oxidative stress markers were analysed. Real time PCR was done to monitor the expression of IRS2 and GRIA2

genes. The LC-ESI/MS analysis showed the presence of various phenolics and flavonoid compounds specific to bud and flower parts. The antidiabetic activity results showed that the animal treated with *C. auriculata* L. bud ethanol extract (CABE500) could better reverse and control the progression of the disease compared to the flower ethanol extract. The gene expression studies revealed that regulation of IRS2 gene occurred in bud but not in flower extract treated animal livers and no differential expression of GRIA2 gene in all the experimental groups. *C. auriculata* L. bud extract can potentially better control the diabetes compared to the flower extract.[36]

Mofizur Rahman,*et al*.,(2011) formulated systems for oral sustained release drug delivery systems using diverse grades of hydroxypropyl methylcellulose (Methocel K4M, K15M, K100M and K100LV), in order to the effect of various grades of these polymer on release mechanism from matrix tablets. Diclofenac Sodium was used as a model drug to evaluate its release characteristics from different matrices. HPMC matrix tablets of Diclofenac Sodium using HPMC (methocel K100LV K4M, K15M, K100M CR) lactose were prepared by direct compression process. The USP paddle method was selected to perform the dissolution profiles carried out by USP apparatus 2 (paddle) at 50 rpm in 900 ml 0.1 N HCl, and phosphate buffer. Drug release was analyzed according to their kinetic models. A One way analysis of variance (ANOVA) was used to interpret the result. Statistically significant differences were found among the drug release profile from different matrices. At a fixed polymer level, drug release from the higher viscosity grades (K100M) was slower as compared to the lower viscosity grades (K100LV). The best-fit release kinetics was achieved with the zeroorder plot, followed by the Higuchi and Korsmeyer equations. Two formulations showed drug release is more controlled. The data obtained proved that the formulations are useful for a sustained release of Diclofenac. From these formulations corresponded more controlled of the drug release by the higher viscosity grade of HPMC. The release of the model drug from these HPMC matrix tablets was prolonged; as a result, an oral release dosage form to avoid the gastrointestinal adverse effects was achieved.[37]

Mubarak Patel, *et al.,(2011)* designed evaluated the microspheres of metoclopramide hydrochloride as a model drug by solvent evaporation method with carbopol and HPMC polymers in various proportions. A total of six formulations were

prepared i.e. F1, F2, F3, F4, F5 and F6. The microspheres were evaluated for micromeritic properties, particle size, % yield, Drug content and Drug release. The size or average diameter of prepared microspheres were recognized and characterized by scanning electron microscopic methods. Microspheres were found discrete, spherical and free flowing. They ranged in particle size from 45.6- 52.2 µm. Metoclopramide hydrochloride release from these microspheres was slowed, extended and depended on the type of polymer used. The formulation F2 and F5 showed consistent drug release for up to 12 h time period. Among all the formulations, F2 contains carbopol 934 and F5 containing HPMC showed the reproducible results with best release profile and good surface morphology. Release data were analyzed based on Highuchi kinetics and Korsmeyer/Peppa's equation and all the selected formulations showed good fit to Peppa's equation. all the formulations of microspheres, particularly those of formulation F2 are promising candidates for the sustained release of metoclopramide hydrochloride in the gastrointestinal tract.[38]

Diptiphadtare, *et al*.,(2014) demonstrated hydroxyl propyl methyl cellulose (HPMC) also know as hypromellose, is largely in used cellulose ether in the development of hydrophilic matrices. Hypromellose provides the release of a drug in a controlled manner, effectively increasing the duration of release of a drug to prolong its therapeutic effect. This provides a current insight into hypromellose and its applicability to hydrophilic matrices in order to highlight the basic parameters that affect its performance. Topics covered include the chemical, thermal and mechanical properties of hypromellose, hydration of the polymer matrices, the mechanism of drug release and the various models used to predict the kinetics and mechanism of drug release from the HPMC matrices. This review also provides the maximum potency of hypromellose used in various dosage form and current patent status review of hypromellose as a release controlling polymer in extended release matrix systems.[39]

Serveh Ghaderi,*et al*.,(2015) described Hydroxypropylmethylcellulose (HPMC) also know as hypromellose, is largely in used cellulose ether in the development of hydrophilic matrices. Hypromellose provides the release of a drug in a controlled manner, effectively increasing the duration of release of a drug to prolong its therapeutic effect. This provides a current insight into hypromellose and its applicability to

hydrophilic matrices in order to highlight the basic parameters that affect its performance. Topics covered include the chemical, thermal and mechanical properties of hypromellose, hydration of the polymer matrices, the mechanism of drug release and the various models used to predict the kinetics and mechanism of drug release from the HPMC matrices. This review also provides the maximum potency of hypromellose used in various dosage form and current patent status review of hypromellose as a release controlling polymer in extended release matrix systems.[40]

Tamizhrasi,*et al*.,(2009) prepared nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate polymethacrylic acid nanoparticles containing lamivudine in different drug to polymer ratio by nanoprecipitation method. SEM indicated that nanoparticles have a discrete spherical structure without aggregation. The average particle size was found to be 121 + 8 - 403 + 4 nm. The particle size of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. The drug content of the nanoparticles was increasing on increasing polymer concentration up to a particular concentration. No appreciable difference was observed in the extent of degradation of product during 60 days in which, nanoparticles were stored at various temperatures. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The *in-vitro* release behaviour from all the drug loaded batches was found tobe zero order and provided sustained release over a period of 24 h. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations and could possibility be advantageous in terms ofincreased bioavailability of lamivudine [41]

Saeed, et al.,(2012) designed gammaoryzanol is a natural antioxidant and could provide beneficial as used in the food products due to the antioxidant activity and potential health benefits. Nano technology has been introduced into several aspects of the food science, including encapsulation of materials and used as delivery systems. The field of nanoparticle delivery systems for nutrients and nutraceuticals has been expanding over the last decades. The aim of this work was evaluation of different methods for preparation of polymeric nanoparticle containing gammaoryzanol.

Nanoprecipitation technique, where polymer and gammaoryzanol were dissolved inacetone, nano-emulsion template method, by stepwise addition of water in to the oilphase consisting of ethyl acetate, gammaoryzanol and surfactant mixtures, as well as emulsification solvent evaporation technique, where gammaoryzanol and polymer were dissolved in ethyl acetate and chloroform, were used for preparation of nanoparticles. Two ratio of gammaoryzanol-polymer (Ethyl cellulose) (1:2 and 1:4) aswell as different solvents and surfactants were used in these methods to produce gammaoryzanol nanoparticles. Among these methods, solvent evaporation technique has been successfully employed to produce gammaoryzanol loaded nanoparticles with desired characteristics [42]

Namitarajput, *etal*.,(2015) formulated nanomaterials and Nanotechnologies attract tremendous attention in recent researches. New physical properties and new technologies both in sample preparation and device fabrication evoke on account of the development of nanoscience. Various research fields including physics, chemists, material scientists, and engineers of mechanical and electrical are involved in this research. Synthesis of nanomaterials that are synonyms to quantum confined atom is an important milestone in the pursuit. Materials scientists and engineers have made significant developments in the improvement of methods of synthesis of nanomaterial solids. In this review various methods of preparing nanomaterials including Gas Condensation, Vacuum Deposition and Vaporization, Chemical Vapor Deposition (CVD) and Chemical Vapor Condensation (CVC), Mechanical Attrition, Chemical Precipitation, Sol-Gel Techniques, Electrodeposition are discussed.[43]

Yadev ,*et al*.,(2012) formulated Polymeric nanoparticles (PNPs) are defined as particulate dispersions or solid particles with size in the range of 10-1000nm. There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size,

biocompatibility with tissue and cells. Several methods to prepare polymeric nanoparticles have been developed and these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer. The different techniques for preparation of polymeric nanoparticles are described. [44]

Abhilash, *et al*.,(2010). studied Nanoparticles (NP) are defined as particles with a diameter smaller than 100 nm, are increasingly used in different applications, including drug carrier systems and to pass organ barriers such as the blood-brain barrier. Because of their unique properties nanocrystals (quantum dots) and other nanoparticles (gold colloids, nanobars, dendrimers and nanoshells) have been receiving a lot of attention for potential use in Therapeutics, Bioengineering and therapeutics drug discovery. In this review potential use of these Nanocrystals and Nanoparticles in various important areas has been discussed.[45]

Sovanlalpal,*et al*.,(2011). prepared special properties of these nanoparticles may offer new advancement in drug discovery. In recent years, there has been an exponential interest in the development of novel drug delivery systems using nanoparticles. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration and the capability to deliver both hydrophilic and hydrophobic drug molecules. This review focuses on classification methods of preparation, characterisation, application, advantages of nanoparticles and health perspectives.[46]

Renu Tiruma, *etal*.,(2015) encapsulated nanotechnology defined as a tiny science. Design characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale is refers to nanotechnology. Nanotechnology by which we can achieve better therapeutic action, better bioavailability and better patient compliance. Several nanoformulations are successfully used for brain delivery which includes nanoparticles system (polymeric/solid lipid), liposomes, dendrimer"s, nanoemulsions, nanosuspension and ligand mediated nanosystems. Nanoparticles are defined as particulate dispersions or solid particles drug carrier that may or may not be biodegradable. Several techniques are used for preparation of

nanoparticles like Solvent Evaporation, Double Emulsification method, Emulsions - Diffusion Method, Nanoprecipitation, Coacervation method, Salting Out Method, Dialysis and Supercritical fluid technology. Nanoparticles are subjected to several evaluation parameters such as yield of nanoparticles, Drug Content / Surface entrapment / Drug entrapment, Particle Size and Zeta Potential , Surface Morphology, Polydispersity index, In-vitro release Study, Kinetic Study, Stability of nanoparticles.[47]

Jafar Akbari,*et al*.,(2011) focused to comparatively evaluate the effect of two hydroxylpropyl methylcellulose (HPMC) molecularweight grades (K4M and K15M) on drug release from diclofenac sodium matrix tablets.Tablets containing diclofenac sodium were prepared by direct compression method at various drug/HPMC ratios and evaluated in vitro for their water uptake, erosion and dissolution characteristics over a period of 8 h. Their release data were analyzed according to various release kinetic models.The release rate of diclofenac decreased with increase in polymer content and was dependenton the HPMC type used, with the lower release rate observed in formulations containing the higher molecular weight grade HPMC K15M. Formulations containing the higher molecular weight HPMC (F4,F5 and F6) showed higher water uptake than those containing the lower molecular weight polymer (F1,F2 and F3) ($p < 0.001$). The formulations incorporating the lower molecular weight HPMC K4M (F1, F2and F3) showed higher erosion than those that contained HPMC K15M (F4, F5 and F6) ($p < 0.001$).Kinetic data based on the release exponent, n, in Peppas model, showed that n values were between0.14 and 0.55, indicating that drug release from HPMC matrices was predominantly by diffusion.This study demonstrates that the molecular weight (MW) of HPMC does affect the water uptake and erosion as well as the rate of drug release from of HPMC matrices.[48]

Chinmaya Keshari Sahoo,*et al*., (2012) evaluated hydroxy propyl methyl cellulose (HPMC)/hypromellose is widely used cellulose ether in the development of hydrophilic matrices for the development of various pharmaceutical dosage forms. HPMC is a polymer selected by most formulators as a hydrophilic matrix system probably due to the claim that it gives fast gel formation to control initial drug release. It is nontoxic, ease of compression and high drug loading capacity. It provides the release of drug in a controlled manner and giving maximum utilization of drug. This review gives

idea about properties of HPMC, mechanism of drug release, factors affecting release from hypromellose and its application. [49]

Fathima M.Hussein*, etal*.,(2017) reviewed the effective surface area of drug particle is increased by a reduction in the particle size. Since dissolution takes place at the surface of the solute, the larger the surface area, the further rapid is the rate of drug dissolution. Ketoprofen is class II type drug according to (Biopharmaceutics Classification System BCS) with low solubility and high permeability. The aim of this investigation was to increase the solubility and hence the dissolution rate by the preparation of ketoprofen nanosuspension using solvent evaporation method. Materials like PVP K30, poloxamer 188, HPMC E5, HPMC E15, HPMC E50, Tween80 were used as stabilizers in perpetration of different formulas of Ketoprofen nanosuspensions. These formulas were evaluated for particle size, entrapment efficiency of drug (EE), effect of stabilizer type, effect of stabilizer concentration and in-vitro dissolution studies. All of the prepared Ketoprofen nanosuspensions formulas showed a particle size result within Nano range. The average particle size of Ketoprofen nanosuspensions formulas was observed from 9.4 nm to 997 nm. Entrapment efficiency was ranged from 79.23% to 95.41 %. The in vitro dissolution studies showed a significant ($p<0.01$) enhancement in dissolution rate of nanosuspension formulas compared to pure drug (drug alone) and physical mixture (drug and stabilizer). Of solvent evaporation method for Ketoprofen with improved in vitro dissolution rate and thus perhaps enhance fast onset of action for drug.[50]

Gunjan Subedi, *et al*.,(2017) studied the methods of formulation of micro and nanospheres such as solvent evaporation, solvent removal, polymerization, hot-melt encapsulation, coacervation, phase/wet inversion, spray drying, spray congealing *etc*. Amongst these all, solvent evaporation is one of the most widely used, researched, easy, accessible methods and for which many patents have been applied. It is thus imperative to understand the basics of effect of formulation variables and design of solvent evaporation method which will be covered in this review article.[51]

Shaikhul Millat Ibn, et al.,(2012) evaluated the effect of water soluble surfactant, sodium lauryl sulphate (SLS) on the release profile of a poorly soluble drug, carbamazepine. Matrix tablets of carbamazepine were prepared by direct compression

method using Methocel K15MCR as release controlling polymer. Varying amounts of SLS were used in seven different formulations to observe the impact on the extent of release rate and mechanism of drug release. Avicel PH101, a derivative of microcrystalline cellulose, and magnesium stearate were used as direct compression diluent and lubricant respectively. The dissolution study of carbamazepine from these extended release matrix tablets was conducted for 8 hours using paddle method in 900 ml 0.1N HCl as dissolution medium. The data obtained form the dissolution studies were explored and explained with the help of zero order, Higuchi and Korsmeyer's kinetic equations. It was found that the dissolution rate of carbamazepine was directly proportional to the amount of SLS present in the matrix tablets. The most important finding was the shift of release mechanism due to the incorporation of a solubilizer. In one end where there was no SLS or smaller amount of SLS, the release mechanism was dominated by polymeric erosion and swelling. On the other hand, drug release mechanism was controlled by Fickian diffusion where SLS content was higher. In addition, under the experimental conditions MDT values declined as the surfactant concentration in the tablet matrices was increased. dissolution rate, extent and mechanism of carbamazepine could be manipulated by optimizing the amount of SLS in the tablet formulation.[52]

Srinivasan, *et al*., (2015), studied the silver nanoparticles were synthesized using flower extract of *Cassia auriculata* as a reducing agent by a simple and eco-friendly route. The aqueous silver ions when exposed to flower broth were reduced and resulted in green synthesis of silver nanoparticles. The reduced silver nanoparticle was characterized using different techniques such as UV-Visible spectrometer, transmission electron microscope (TEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FT-IR) analysis. Furthermore the novel approach to analyze the antimicrobial activity against human pathogenic organisms through biosynthesized silver nanoparticles.[53]

Jyothi., et al.,(2012). designed to assess *in vitro* and *in vivo* antioxidant and antidiabetic efficiency of *Cassia auriculata* L. flowers and its phytochemical analysis. *In vitro* antioxidant competence was screened for scavenging DPPH, superoxide, nitric oxide, hydroxyl, H O and lipid peroxides 2 2 in addition to reducing power and metal ion

chelating capabilities. The inhibitory effect on carbohydrate digestive enzymes -amylase and -glucosidase was studied with reference to acarbose. *In vivo*, parameters such as fasting blood glucose,glycosylated hemoglobin, plasma insulin, reduced glutathione and activities of antioxidant enzymes were studied with reference to glibenclamide. The methanolic extract showed higher antilipid peroxide and DPPH radical scavenging ability. The potent inhibitory effect was observed on activities of -amylase and -glucosidase. The marked decrease in the glucose level in the extract treated streptozotocin induced diabetic rats was due to elevated levels of insulin. The increased activity of antioxidant enzymes and glutathione, accounts the antiradical activity of the extract. RP-HPLC analysis indicated the presence of mixed catechins, caffeine and quercetin. These bioactive constituents validate antioxidative ability and provides scientific basis for the usage of *Cassia auriculata* L. flowers in ayurvedic formulations in the treatment of diabetes and other related inflammatory diseases.[54]

Fatimah M. Hussein Wais, *et al*.,(2017) formulated effective surface area of drug particle is increased by a reduction in the particle size. Since dissolution takes place at the surface of the solute, the larger the surface area, the further rapid is the rate of drug dissolution. Ketoprofen is class II type drug according to (Biopharmaceutics Classification System BCS) with low solubility and high permeability. The aim of this investigation was to increase the solubility and hence the dissolution rate by the preparation of ketoprofen nanosuspension using solvent evaporation method. Materials like PVP K30, poloxamer 188, HPMC E5, HPMC E15, HPMC E50, Tween 80 were used as stabilizers in perpetration of different formulas of Ketoprofen nanosuspensions. These formulas were evaluated for particle size, entrapment efficiency of drug (EE), effect of stabilizer type, effect of stabilizer concentration and in-vitro dissolution studies. All of the prepared Ketoprofen nanosuspensions formulas showed a particle size result within Nano range. The average particle size of Ketoprofen nanosuspensions formulas was observed from 9.4 nm to 997 nm. Entrapment efficiency was ranged from 79.23% to 95.41 %. The in vitro dissolution studies showed a significant ($p<0.01$) enhancement in dissolution rate of nanosuspension formulas compared to pure drug (drug alone) and physical mixture (drug and stabilizer). Solvent evaporation method for Ketoprofen with

improved in vitro dissolution rate and thus perhaps enhance fast onset of action for drug [55]

Kavimani, *et al*., (2015) evaluated the *In Vitro* Antibacterial activity of crude extract of locally available plant Cassia auriculata flowers**.** The current study was performed to screen the phytochemicals that are present in *C. Auriculata* flowers. To prepare the extract, the shade-dried flowers of C. Auriculata were soaked in water, petroleum ether and methanol. The C. Auriculata flowers extract has several bioactive compounds.The TLC technique has been used to identified the possible compounds present in the methanol extract. The FT-IR spectral data shows functional groups of possible chemical compounds present in the methanol extract of *C.Auriculata*. The extract was subjected to Disc Diffusion Method to find out the biological activities with three different concentrations [50, 75,100 µl/ml].The methanol extract was only used for this study by using Disc Diffusion [56]

Chinmaya Keshari Sahoo, *et al*., (2015) studied the hydrophilic matrices are typically compressed (Hogan, 1989) powder mixtures of drug and excipients including one or more water swellable hydrophilic polymers which are generally regarded as safe (GRAS) excipients. Hydrophilic matrices use polymers with flexible chemistry that offers an opportunity to design controlled release dosage forms for wide range of drugs with varying solubility and doses. Swellable matrices can be administered in various routes such as oral, buccal, vaginal, rectal drug delivery system. There are many high molecular weight water soluble or water swell able polymers used in hydrophilic matrices such as HPMC, hydroxyl propyl cellulose, sodium carboxy methyl cellulose, sodium alginates, carbomer etc. HPMC is most popular polymer in matrix applications because of its ability to obtain desired release profiles for wide range of drugs, provides robust formulation, global availability, cost effective manufacture, broad regulatory acceptance etc. HPMC is typically used as primary polymer and other polymers which can modulate the drug release profile in controlled manner (Fallingborg, 1999). Solubility of drug determines the mechanism of drug release from HPMC hydrophilic matrices influencing the choice of polymer viscosity, chemistry and other excipients. Use of an appropriate viscosity grade will enable a formulation scientist to design matrices based on diffusion, erosion mechanism etc. Practically insoluble drugs

dissolve slowly and have slow diffusion through gel layer of a hydrophilic matrix. The aim of this review is to give idea into hypromellose, mechanism of drug release, factors affecting release from hypromellose and its application.[57]

V Selvi, et al., (2015). Investigated the medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. The leaf and flower extracts were subjected to qualitative chemical screening of active chemical constituents. Preliminary phytochemical analyses of different extracts were carried out. In *Cassia fistula* the methanol extract results were positive for alkaloids, phenols, glycosides and tannins, Chloroform extract showed positive test for saponins, carbohydrates, glycosides and tannins. In *Cassia auriculata* extract were positive test for alkaloids, flavonoids, triterpenoids, glycosides, tannins, amino acids and saponins where as aqueous extract was found to be positive for flavonoids, alkaloids, carbohydrates, glycosides, amino acids and saponins. These secondary metabolites are the active constituents of *Cassia fistula* and *Cassia auriculata.*[58]

Nagavarma, *et al*., (2012) studied polymeric nanoparticles (PNPs) are defined as particulate dispersions or solid particles with size in the range of 10-1000nm. There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells. Several methods to prepare polymeric nanoparticles have been developed and these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer.[59]

J. Syam Praveen Kumar, et al., (2014) designed to explore the protective effect of *Cassia auriculata* L. flower extract (CAE) in high fat diet and streptozotocin induced type 2 diabetic (T2DM) rats. T2DM was induced by a combination of high fat diet and low dose streptozocin. Rats in different groups were treated with *Cassia auriculata* L. flower extract at two different doses viz. 300mg and 500mg/Kg body weight and the hypoglycemic potential as well as lipid lowering and antioxidant properties of CAE in liver and pancreas were evaluated. T2DM rats showed significantly elevated glucose and reduced c-peptide levels in serum. Also there was significant increase in serum marker enzymes of liver toxicity-alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase (ALP) along with significant reduction in liver glycogen and increase in lipid peroxidation levels. There was also deregulation in lipid levels in plasma and liver and significant reduction in antioxidant enzymes in plasma, liver and pancreas. Encouragingly, treatment with *Cassia auriculata* extract caused significant improvement in the glucose, insulin, lipid levels in plasma and the antioxidant status of liver and pancreas.levels of antioxidants and lipid levels in plasma, liver and pancreas indicating its potential to ameliorate peripheral insulin resistance.[60]

Mohamed Sham Shihabudeen., *et al.,* (2010) studied the success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains. Methanol extracts of six plant species traditionally used in Indian folklore medicine for the treatment of various bacterial and fungal infections were investigated for *in vitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* by disc diffusion method. Methanol extracts of *Eugenia jambolana* and *Cassia auriculata* showed the highest toxicity against all the bacteria. The plant extracts showed antibacterial activity but not antifungal activity against any of the fungi used. Minimum inhibitory concentration (MIC) assay were determined for these two extracts against bacteria. *E. jambolana* revealed the highest antimicrobial activity at a minimum concentration (0.75 mg/ml) against *S. aureus.* The phytochemical analysis carried out revealed the presence of coumarins, flavanoids, glycosides, phenols, tannins, saponins and steroids**.**

Alkaloids were not detected from any of the plant extracts under study. Use of the plants in folk medicine to treat various infectious diseases [61]

Sergio Freitas., ***et al.,*** **(2004)** formulated the therapeutic benefit of microencapsulated drugs and vaccines brought forth the need to prepare such particles in larger quantities and in sufficient quality suitable for clinical trials and commercialisation. Very commonly, microencapsulation processes are based on the principle of so-called bsolvent extraction/evaporationQ. While initial labscale experiments are frequently performed in simple beaker/stirrer setups, clinical trials and market introduction require more sophisticated technologies, allowing for economic, robust, well-controllable and aseptic production of microspheres. To this aim, various technologies have been examined for microsphere preparation, among them are static mixing, extrusion through needles, membranes and microfabricated microchannel devices, dripping using electrostatic forces and ultrasonic jet excitation. This article reviews the current state of the art in solvent extraction/evaporation-based microencapsulation technologies. Its focus is on process-related aspects, as described in the scientific and patent literature. Our findings will be outlined according to the four major substeps of microsphere preparation by solvent extraction/evaporation, namely, incorporation of the bioactive compound, formation of the micro droplets,solvent removal and (iv) harvesting and drying the particles. Both, well-established and more advanced technologies will be reviewed [62]

S. Bhatia.,*et al., (*2016). evaluvated most emerging branch in pharmaceutical sciences known as " P*harmaceutical nanotechnology* " presents new tools, opportunities and scope,which are expected to have significant applications in disease diagnostics and therapeutics. Recently nano-pharamceuticals reveal enormous potential in drug deliveryas carrier for spatial and temporal delivery of bioactive and diagnostics. Additionally it also provides smart materials for tissue engineering. This discipline is now well- established for drug delivery, diagnostics, prognostic and treatment of diseases through its nano engineered tools. Some nanotech based products and delivery systems are already in market. Pharmaceutical nanotechnology comprised of nano- sized products which can be transformed in numerous ways to improve their characteristics. Drugs that are transformed in to nano range offer some

unique features which can lead to prolonged circulation, improved drug localization, enhanced drug effi cacy etc. Various pharmaceutical nanotechnology based systems which can be termed as nano pharmaceuticals like polymeric nanoparticles, magnetic nanoparticles, liposomes, carbon nanotubes, quantum dots, dendrimers, metallic nanoparticles, polymeric nanoparticles, etc. have brought about revolutionary changes in drug delivery as well as the total medical service system. With the aid of nanopharmaceuticals, Pharmaceutical nanotechnology could have a profound infl uence on disease prevention to provide better insights into the molecular basis of disease. However some recently found health risk evidences limits their utilization in pharmaceutical industry. Some concerning issues like safety, bioethical issues, toxicity hazards, physiological and pharmaceutical challenges get to be resolved by the scientists. Current researchers are still lacking suffi cient data and guidelines regarding safe use of these nanotechnology based devices and materials. Therefore pharmaceutical nanotechnology is still in infancy. Types of nanopharmaceuticals with the most important Applications and nanoparticles associated health risk related information available till present.[63]

S.Sankaraiah, *et al*.,(2017) studied (*Cassia auriculata* linn) flowers are used for various conditions of ailments in traditional systems of medicine since ancient times. This study is designed to lay down the various pharmacognostic and phytochemical standards which will be helpful to ensure the purity, safety, and efficacy of this medicinal plant and Various methods including macroscopic, microscopic, physicochemical and phytochemical methods were applied to determine the diagnostic features for the identification and standardization of intact and powdered drug of *Avartaki* (*Cassia auriculata* Linn) flowers. The shape, color, odour and surface characteristics were determined for the intact drug and powdered materials of *Avartaki* (*Cassia auriculata* Linn) flowers. Light and electron microscope images of cross-section of stamen and powdered microscopy revealed useful diagnostic features. Phytochemical, physicochemical analysis of powdered drug proved useful to differentiate the powdered drug material. High performance thin layer chromatography analysis showed the presence of important phytoconstituents. Morphology as well as various

pharmacognostic aspects of different parts of the plant were studied and have been described here along with phytochemical and physicochemical studies, which will help in authentication and quality control.[64]

Rahul. Deshpande., *et al.*, (2011) investigated herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practioners of traditional medicine. In this study the acetone extract of Cassia Auriculata is investigated for its antimicrobial activity against salivary microflora. The salivary samples were collected from children of mixed dentition age group having DMFT 4 and above 4. The microbial inhibition assay was done by 'well diffusion method' on the Muller-Hinton agar. The results were compared with 2% chlorhexidine a known commercial available antimicrobial agent. The results confirmed the antimicrobial potential of this extract and indicated that the acetone extract of Cassia Auriculata can be used as the preventive tool for dental caries.[65]

AIM OF THE WORK

- Traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India; even among those to whom western medicine is available, the number of people using one form or another of complementary of alternative medicine is rapidly increasing worldwide
- Increasing knowledge of metabolic process and the effect of plants on human physiology has enlarged the range of application of medicinal plants. Nearly 50% of medicines in the market are made of natural basic materials
- Interestingly, the market demand for medicinal herbs is likely to remain high because many of the active ingredients in medicinal plants cannot yet be prepared synthetically.
- The World Health Organization (WHO) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary healthcare needs
- In almost all the traditional medical systems, the medicinal plants play a major role and constitute their backbone.
- Indian material medical includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs
- Ant diabetic activity, In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered.
- Persistent hyperglycemia is a major contributor to such metabolic alterations, which lead to the pathogenesis of diabetic complications.
- *Cassia auriculata* flower extract on hepatic glycolytic and gluconeogenic enzymes and STZ-diabetic rats were given the plants extracted per for 30 days.
- An antihyperglycemic effect and suggested that enhanced gluconeogenesis during diabetes is shifted towards normal and that the extract enhanced the utilization of glucose through increased glycolysis. The effect of the extract was more prominent than that of glibenclamide.
- The incidence of diabetes is growing rapidly both in the United States and worldwide. For example, it is estimated that more than 180 million peoples are affected with diabetes, and also prevalence will be expected to more than double

by the year of 2030. In the United States, approximately 21 million peoples are estimated to suffer from diabetes, and it is a major cause of morbidity and mortality.

- Diabetes is heterogeneous group of syndrome characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin.
- In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Over the last few years, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases.
- Similarly it has been already proved that various parts of plants such as Leafs, fruits, seeds etc. provide health and nutrition promoting compounds in human diet.
- The *Cassia auriculata Linn* is another Indian plant, which has enormous traditional uses against various diseases. The present review aims to compile medicinal values of *Cassia auriculata.*Linn generated through the research activity using modern scientific approaches and innovative scientific tools.
- *Cassia auriculata* is one of the most traditionally using hypoglycemic agents among tribes in India and it is not
- Scientifically validated. Based on the above mentioned reasons, one new research is required to develop one new drug with more anti diabetic activity

Aim of the current work is

1. Formulation and evaluation of *Cassia auriculata*.L Nanoparticles
2. To study the anti diabetic activity of developed activity.

PLAN OF THE WORK

- Collection and drying the flower of plant *Cassia auriculata*.L
- Pharmacognostic studies of plant *Cassia auriculata*.L
    - Macroscopical character
    - Microscopical character
- Soxlet alcoholic extract of *Cassia auriculata*.L
- Phytochemical estimation of plant *Cassia auriculata*.L
- Formulaion of *Cassia auriculata*.L nanoparticles
- Evaluation of *Cassia auriculata*.L nano particles
    - Drug entrapment effiency of *Cassia auriculata*.L nanoparticles
    - FT-IR of *Cassia auriculata*.L nanoparticles
    - *In vitro* release study of *Cassia auriculata*.L nanoparticles
    - Stability studies of *Cassia auriculata*.L nanoparticles
    - SEM of *Cassia auriculata*.L nanoparticles
    - Kinetics of drug release of *Cassia auriculata*.L nanoparticles
    - Anti diabetic activity of *Cassia auriculata*.L nanoparticles

4.1. List of Instruments Used

S.No	Materials	source
1	*Cassia auriculata.*L.Flower	vagaikulam, Thoothukudi
2	Hydroxyl propyl methl cellulose	S.D.Fine Chem Ltd.Boisar
3	Sodium lauryl sulphate	S.D.Fine Chem Ltd.Boisar
4	Methanol	S.D.Fine Chem Ltd.Boisar
5	Chloroform	S.D.Fine Chem Ltd.Boisar
6	Methanol	S.D.Fine Chem Ltd.Boisar

4.2 List of Chemical Used

S.No	Equipments	Company
1	Rotary flash evaporater	Equitron, Mumbai
2	Probe sonicater	Bandelin,HD2070 GERMANY
3	UV- Visible sectrophotometer	Shimaducorporation, Japan
4	Stability chamber (120 liters)	Osworld,JRIC-11 MUMBAI
5	Single pan electronic balance	Shimadzu
6	Magnetic stirrer	Remi motor Ltd,CM101 Mumbai
7	pHmeter	ELICO, Pvt, Ltd. L1127, Chennai.
8	Autoclave	Dalal, Chennai.
9	Laminar air flow bed	Klenzoids.Mumbai.
10	Hot air oven	Biochem, Mumbai.
11	IR Spectrometer	Perkin Elmer, Germany
12	Cooling centrifuge	Remi motor Ltd, Mumbai

4.3 PLANT PROFILE [66]

Botanical Name: *Cassia auriculata .L*

Fig no: 3. *Cassia auriculata* .LTree

Taxonomy:

Table 1. Morphology chararter of flower *Cassia auriculata*

Kingdom :	Plantae
Order:	Plantae
Family:	Fabaceae
Genus:	Cassia
Spices	Auriculata

Vernacular Names:

Table 2. Vernacular Names of flower *Cassia auriculata*

English	Tanner's Cassia
Ayurvedic	Aaavartaki, Aaadaari
Unani	Tarwar
Siddha/ Tamil	Aavaarai
Folk	Tarwar
Hindi	Awal ,Tarwar

Botanical Description:

The leaves:

Are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at base on side of next petiole into a filliform point and persistent.

Flowers:

Irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules.

The fruit:

A short legume, 7.5–11 cm long, 1.5 cm broad, oblong, obtuse, tipped with long style base, flat, thin, papery, undulately crimpled, pilose, pale brown. 12-20 seeds per fruit are carried each in its separate cavity

Habitat:

It is distributed throughout hot deciduous forests of India. Wild in dry regions of Madhya Pradesh, Tamil Nadu Rajasthan and other parts of India.

Parts Used:

Leaves, flower, fruit, bark, roots, seeds.

Fig no : 4 *Cassia auriculata* L.flower

Cultivation and collection: [67]

A plant mainly of dry regions in the tropics, though it is also able to tolerate much wetter conditions. It grows best in areas where the mean annual temperature is in the range 16 - 27Â°c and can tolerate a mean annual precipitation as low as 400mm, or up to 4,300 mm Requires a position in full sunTolerant of many soil types, including saline, but prefers a fairly rich, well-drained soilPlants are fairly fast-growing and can reach a height of about 3 metres with 35mm stem diameter within 2 years; and a height of about 5 metres with 70mm stem diameter within 4 years Plants respond well to coppicing Plants can be harvested for tannins and dyestuff from the third year onwards, with the plants being coppiced annually Flowering and fruiting is often almost throughout the

year, though there can be periods of increased flowering at times of the monsoon although many species within the family Fabaceae have a symbiotic relationship with soil bacteria, this species is said to be devoid of such a relationship and therefore does not fix atmospheric nitrogen

Medicinal uses: [68]

- Roots- used in skin diseases and asthma.
- Flowers used in diabetes, urinary disorders and nocturnal emissions.
- Its Bark is used as astringent.
- Leaves and Flowers – Anti-diabetic activity
- Some caution should be exercised when eating this plant due to reports of toxicity
- Young leaves - occasionally eaten
- The leaves are made into a refreshing drink
- The dried leaves are used to make a tea
- Young pods - occasionally eaten
- Young flowers - occasionally eaten
- The dried flowers are used to make a coffee substitute
- A fermented mixture of pounded bark and dissolved molasses is used as an alcoholic beverage

Flowers: Urinary discharges, diabetes, throat irritation

Bark: Astringent, haemorrhage

Leaf: Induced alcohol related liver damage, emollient effect

Seeds: Conjunctiva astringent, sour, cooling, constipating, depurative.

Flowers: Antihelmintic

The plant has been taken as medicament by tribal in following methods:

- The bark yields tannin The bark of plants 3 years old or more contains 15 - 24% of tannin on a dry weight basis
- A black dye is obtained from the bark

- A fast yellow dye is obtained from the flowers
- The flower buds are used in the galling process prior to dyeing cotton cloth and chintzes red, pink or purple with madder roots
- The boiled seeds are an important ingredient in indigo vats, where specific bacterial fermentation ensures the reduction of insoluble indigo into the soluble leuco-indigo, allowing textile fibres to be impregnated by the dye solution.
- The seeds serve as a source of sugars to keep the fermentation process going A fibre is obtained from the inner bark
- The bark fibre can be made into rope
- Branches are used as chewing sticks and toothbrushes
- The wood does not reach a volume adequate for timber, but sometimes handles of small tools are made from it
- A most curious use of the plant is reported from India. It is believed that branches were formerly used in the fabrication of wootz Damascus steel.
- They were added to the crucible and heated with the ore to obtain the chemical composition that gave the steel its beautiful patterning

Adverse Effects:

Phytochemical Constituents:[68]

- Pod husk contains nonacosane and nonacosan-6-one, chrysophanol, emodin and rubiadin, β-sitosterol, polysaccharides, flavonoids, anthracene derivatives and some dimericprocyanidins , Saponins and tannins.
- found fatty acid esters, fatty acid amide, terpenoids, diterpene alcohols, phytols as major compound groups in the methanol fractions from the seed extract of Cassia auriculata by GC- MS analysis.
- The chemical composition of the leaves of Cassia auriculata was revealed the presence of 3-O-Methyl-d-glucose (48.50%), α-Tocopherol-β-D mannoside (14.22%), Resorcinol (11.80%), n- Hexadecanoic acid (3.21%), 13-Octadecenal, (Z)- (2.18%) and 1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid (1.98%) which were identified by GC – MS analysis.

- The isolated an antibacterial compound - Oleanolic acid from the leaves of Cassia auriculata and identified by IR spectrum,CNMRHalade investigated the flowers of Cassia auriculata the presence of anthroquinones, aloe emodin and sitosterols

Ethnobotanical uses:

- The plant has been reported to possess antipyretic hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic and microbicidal activity.
- Cassia auriculata has been shown to antiviral activity and anti spasmodic activity.
- The plant is used in the traditional system of medicine for female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam.
- The plant has been widely used as a cure for rheumatism and conjunctivitis.
- The various parts of the plant were reported to exert a beneficial effect to alleviate the symptoms of diabetes
- The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation.
- The Bark is used in skin conditions; bark as astringent, useful in checking secretion or haemorrhage. They also restore the disordered processes of nutrition.
- The Leaf extract has a protective action against alcohol induced oxidative stress to the cells as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymatic and non-enzymatic antioxidants and experimentally induced alcohol related liver damage.
- The leaf extracts also shows emollient effect
- The seeds of tanner's cassia find their application in purulent opthalmia i.e., inflammation of the eye or conjunctiva.
- They should be finely powdered and blown into the affected eyes.
- Seeds are astringent, sour, cooling, constipating, depurative, aphrodisiac, anthelmintic, stomachic, alexeteric, useful in diabetes, chyluria, ophthalmic, dysentery, diarrhoea, swellings, abdominal
- disorders, leprosy, skin diseases, worm infestations, chronic purulent conjunctivitis .

- The Roots are used in skin diseases and asthma.
- The roots are astringent, cooling, alterative, and depurative and alexeteric, and are useful in skin diseases, leprosy, tumors, asthma and urethroroea.
- Leaves, Flowers and Fruits as antihelmintic; its leaves and petals are both mildly astringent in taste. It also checks the flow of extra amount of urine and helps in absorption of required amount of fluids in the kidneys and intestines.
- The anti-inflammatory activity of various extracts of leaves was carried out using carrageenan induced rat paw edema.

4.4. EXICIPIENT PROFILE[70]

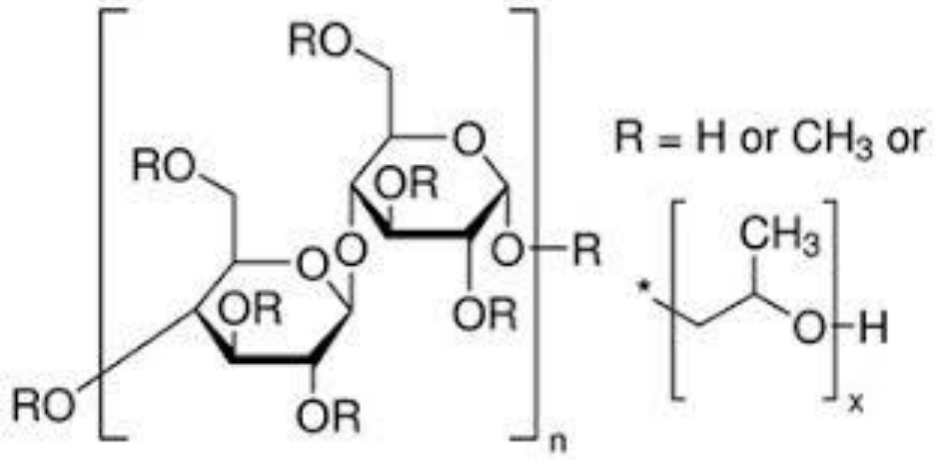
4.4.1. HPMC:

Hydroxy propyl methyl cellulose

Molecular Weight:

324.2848

Chemical Structure:



Hydroxy propyl methyl cellulose (HPMC):

92 R is H, CH3, or CH3CH (OH) CH2

Structural formula Functional Category:

Coating agent; extended release agent

Applications: Tablet binder, in film-coating, and as a matrix for use in extended-release tablet formulations.

Description: Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder.

Glass transition temperature: 170–180°C.,

Melting point: 190–200°C.

Solubility:

Soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Few grades of HPMC are soluble in acetone, mixtures of dichloromethane and propanol, and other solvents.

Viscosity:

Wide range viscosity grades are available in the market.

Stability and Storage Conditions:

Hypromellose powder is a stable material, although it is hygroscopic after drying.

4.4.2.Sodium Dodecyl Sulfate (SDS), Lauryl [71]

Molecular Weight: 288.5g

Chemical Structure:

Chemical Name:

Sodium deodecylsulphate, 151-21-3;

SODIUM LAURYL SULFATE;

Sodium dodecylsulfate,

Sodium lauryl sulphate,

Dodecyl sodium sulfate

Molecular Formula: C12H25NAO4S

Physical Description:

SODIUM DODCYL SULPHATE, SODIUM SALT] is a white to pale yellow paste or liquid with a mild odor. Sinks and mixes with water

Color:

White or cream-colored crystals, flakes, or powder

Solubility:

less than 1 mg/mL at 66° F

Physical properties:

- Sodium dodecyl sulfate is an anionic surfactant, and is a typical representative of sulphate-based surfactant.
- It is abbreviated as SDS, and also known as AS, K12, coco alcohol sulfate, sodium lauryl sulfate and foaming agent.
- The commercial products are usually white to light yellow crystalline powder.
- It is non-toxic, slightly soluble in alcohol, insoluble in chloroform and ether, soluble in water, and has good anionic and nonionic complex compatibility.
- It has good emulsibility, foamability, and foaming, infiltrating, decontaminating and dispersing properties.
- It is abundant in foams

Uses: [72]

- Sodium dodecyl sulfate has excellent detergency,
- Emulsification and foaming power, can be used as detergents and textile auxiliaries
- Used as an anionic surfactant
- Toothpaste foaming agent,
- Mine fire extinguishing agents,

5.1. Collection of Flower *Cassia auriculata*.L: [73]

The fresh flower of *Cassia auriculata*.L.was collected in the month of November from vagaikulam, thoothukudi, tamilnadu. The plant was identified and authenticated by The American College, Madurai. The fresh bark was used for the study of macro morphological and microscopy, physicochemical characterization and phytochemical analysis.

5.1.1. Macroscopic characters of Flower *Cassia auriculata*.L [74]

The flower of cassia auriculata was subjected to macroscopic studies which comprised of organoleptic characteristics viz.colour, odour, appearance taste, shape, texture, fracture, etc. of the drug. These parameters are considered as quite useful in quality control of the crude drud and where evaluated as per standard WHO guidelines

Table 3. The Observation has been tabulated in the following

Shape	slender
Margin	few-flowered
Colour	bright yellow
Taste	Astringent
Odour	characteristics

Irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules.

5.1.2. Microscopic Characteristics Flower of *Cassia auriculata*.L [75]

Fresh flowers of cassia auriculata were selected for the microscopically studies. Microscopic sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the microscopically sections of the bark specimen were made and examined microscopically. Histochemical reactions and on flower specimen were made and examined microscopically. Histochemical reactions were applied with staining reagents on transverse sections and on bark powder by reported methods. Photomicrographs of the microscopically sections were taken with the help of Microscope.

Powder Characteristics Flower of *Cassia auriculata*.L: [76]

Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination was carried out as per reported methods.

5.1.3. Physicochemical Evaluation Flower of *Cassia auriculata*.L: [77]

Analysis of Physiochemical constants of the powder flower has been done to evaluate the quality and purity of the drug. Various physochemical parameters like moisture contents, foreign organic matters, Ash values and Extractive values ware calculated as per WHO guidelines. The information collected from these test was useful for standardization and obtaining the quality standards.

5.1.4. Phytochemical Investigations Flower of *Cassia auriculata*.L: [78]

The qualitative chemical tests carried out for the identification of the nature different phytoconstituents present in the powered crude drug the tests were carried out by using standard conventional protocols.

Extraction Process of Flower *Cassia auriculata*.L: [79]

Cassia auriculata .L flowers (500g) were extracted with1500 ml of Ethanol by the method of continuous hot extraction at 60 °C for 6 h and evaporated. The residual extract was dissolved in water and used in the study

Determination of Absorbance maximum ((λ_{max}) [80]

Cassia auriculata. L was dissolved in phosphate buffer saline7.4 pH, solution with 20 µg / ml concentration was prepared by suitable dilution The *Cassia auriculata* .L drug in solution was scanned in UV spectrophotometer from 200 to 400 nm using phosphate

buffer saline pH 7.4 as blank. Absorbance maximum was determined as 284 nm. The drug was later quantified by measuring by measuring the absorbance at 284nm in phosphate buffer saline pH 7.4.

Standard Curve for *Cassia auriculata* .L (by UV method) [81]

Preparation of primary stock solution

Cassia auriculata .L 100 mg weighed and dissolved in phosphate buffer saline p H 7.4 in 100 ml volumetic flask. The flask was shaken and volume was made up to mark with phosphate buffer saline pH 7.4 to give a solution containing 1000µg /ml.

Preparation of secondary stock solution

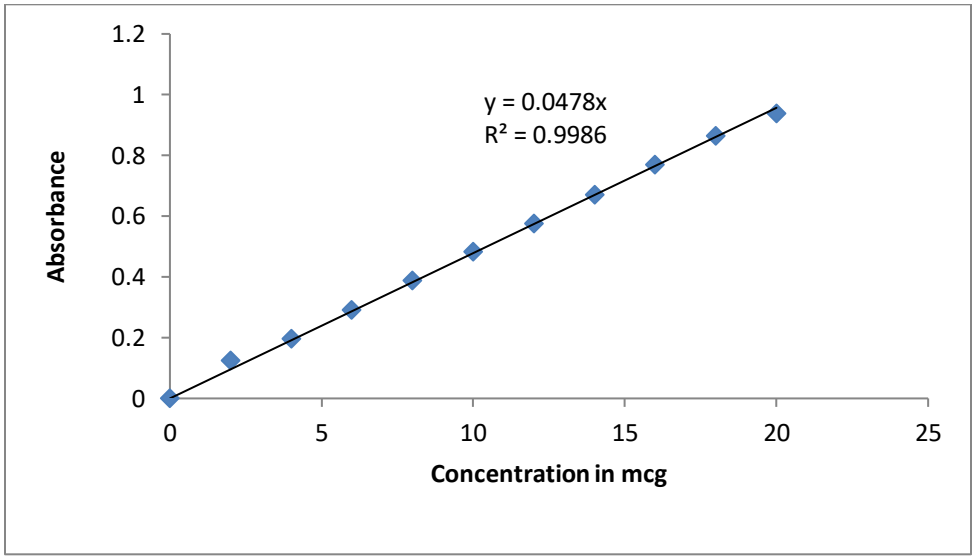
From the primary stock solution, pipette out 2 ml and placed into 100 ml volumetric flask. The volume was made up to mark with phosphate buffer saline pH 7.4 to give a stock solution containing 20 µg /ml

Preparation of sample solution [82]

Appropriate volumes of aliquots (1to10ml) from standard *Cassia auriculata*.L secondary stock solution were transferred to different volumetric flasks of 10 ml capacity The volume was adjusted to the mark with phosphate buffer saline p H 7.4 to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg /ml . Absorbance of each solution against phosphate buffer saline p H 7.4 AS blank were measured at 284 nm and the graph of absorbance against concentration were plotted and shown in Figure .3.

Table. 4. Standard curve data for Cassia auriculata.L alcoholic extract (By U.V method)

Concentration in µg /ml	Absorbance at 284 nm
2	0.125
4	0.197
6	0.291
8	0.389
10	0.483
12	0.576
14	0.670
16	0.769
18	0.865
20	0.939



5.2. Formulation of *Cassia auriculata*.L Extract Nanoparticles:[82]

Emulsion Solvent by Evaporation Method

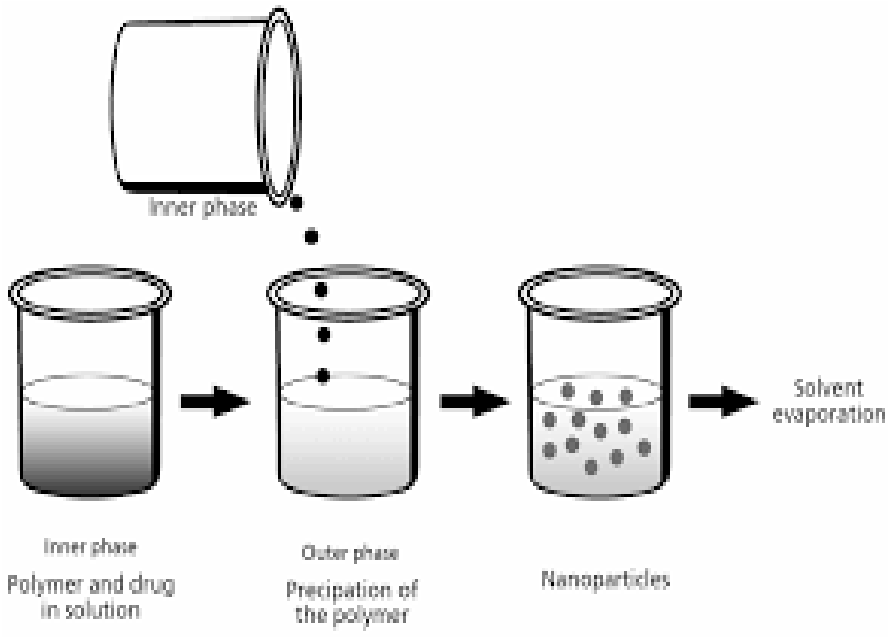
The emulsion thus formed was further evaporated by flash rotatary evaporator for 20 min. The nanoparticle was All batches of nanoparticles were prepared by Emulsion Solvent Evaporation Method. The required quantity of polymer was dissolved in 2.5 ml of water and 2.5 ml of chloroform in (1:1) ratio as organic phase. The organic phase was then mixed with an aqueous phase containing drug and 0.2% polyvinyl alcohol (4ml). The polymer concentration differs in various batches formulation as given in table: 2

This mixture was homogenized by vortex mixture for 1 min and then sonicated using a probe set at 55w collected by ultra-centrifugation (15000rpm)

Table 5: Various Composition of nanoparticles formulation

S.NO	FORMULATION CODE	CASSIA AURICULATA.L	POLYMER HPMC	POLYMER HPMC
1	F1	200	300	-
2	F2	200	200	-
3	F3	200	100	-
4	F4	200	-	300
5	F5	200	-	200

Fig: 5 Schematic representation of emulsion Solvent Evaporation Method



The prepared nanoparticles are washed with Distilled water .The washed liquid was eliminated by centrifugation and purified nanoparticle was collected.

5. 3.Evaluvation of Cassia auriculata.L nanoparticles:[83]

5.3.1. Fourier Transformer Infrared (FTIR) spectral study:

IR study was carried out for identification of pure drugs. IR spectroscopy (using Perkin Elmer) by KBr Pellet method was carried out on drug. They are compressed under 15 tons pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1}in a spectrometer and peaks obtained were identified

Infrared (I.R.) spectrum of drug, physical mixture of drug-polymer loaded microsphere gives information about the group present in that particular compound. Before I.R. spectra studies, Physical mixture of drug-polymer and microsphere were dried in vaccum for 12 hours. Potassium bromide (KBr) 200mg in 3mg test sample was used to prepared discs, scan under the range 4000 – 400 wave number (cm-1) and % Transmittance employing Perkin Elmer (USA). The above experiments were performed in triplicate manner to confirm the results.

Particle size analysis and Scanning Electron Microscopy (SEM) study:[84]

The particle size of microspheres were determined using Scalar-USB Digital scale ver. EPhoto microscope, attached with canon camera system based on mean diameter and then calculated size distribution. The surface morphology and shape of microspheres were analyzed by a Scanning Electron Microscopy (SEM, Hitachi Model S-3000H, CECRI, Karaikudi, Tamilnadu, India). During the SEM examination, a drop of microspheres dispersion to be examined was mounted over a SEM stub and dried in desicator. Microspheres were coated with very thin coat of gold employing a vaccum evaporator to make electrically conductive. Then the size of the microspheres was recorded under SEM at a magnification ranging from 500X to 3000X and operated at an accelerating voltage of 20 kV.

Particle Size and Zeta Potential[85]

Value of Particle size and Zeta Potential prepared nanoparticles determined by using Malvern Zetasizer (Ver. 6.11In SAS process liquid solvents are used, which should completely miscible with the supercritical fluid. The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, it results the formation of nanoparticles. In RESS high degree of super saturation

occure by dissolving solute in a supercritical fluid to form a solution, followed by the rapid expansion of the solution across an orifice or a capillary nozzle into ambient air by the rapid pressure reduction in the expansion which results in homogenous nucleation and thereby, the formation of well-dispersed particles.

Differential Scanning Calorimetry (DSC) study:[86]

The thermal behavior of ACF, physical mixture of drug-polymer and drug-loaded microspheres were investigated employing differential scanning calorimeter (DSC-60 Instruments, Shimadzu Corporation,Japan). The samples (5mg) were accurately weighed, sealed hermetically into aluminum pans and heating run for each sample kept from 50°C- 300°C at a heating rate of 10°Cper min, using in atmosphere of air as blanket gas.

5.4. *In vitro* Drug Release Profile:[87]

The *in vitro* dissolution studies were carried out in phosphate buffer solution (PBS), 900 mL of pH 7.4,maintained at 37 ± 0.5°C temperature thermostatic controlled water bath, 100 rpm by employing basket-type dissolution apparatus (United States Pharmacopeia XXIV) of eightstation (Electro-lab, Mumbai, India). Microspheres weighed contain 200 mg of were used as test sample. Withdrawn the sample solution (5ml) at predetermined time intervals over a period of 12hours, filtered through a 0.45 mm membrane filter,diluted suitably, and assessed for drug release at 284nm for ACF by using a UV spectrophotometer(Shimadzu UV-1700, Japan). After each withdraw, Immediately supplemented an equal amount of fresh PBS. Each determination was performed thrice and the percent cumulative drug release plotted as the percent drug release in dissolution media Vs time

5.5. Stability Studies[88]

The optimized formulation F3 was subjected to stability study for one month at 4 ^{o}C (refrigerator) room temperature and at 45 ^{o}C / 75% RH. At the interval of 30 days, samples of nanoparticles formulation were taken and evaluated for the entrapment efficiency and *in vitro* release of drug (Table 17 to 20). Entrapment efficiency of optimized formulation F9 kept at 9^{o} C shows a release rate of 95.5% after 30 days of stability study .The entrapment efficiency of formulation got decreased on exposure to higher temperature. The percentage entrapment of formulation kept on room

temperature and at 45^{o}C /75% RH were 82% 81% respectively after 30 days of stability study.

5.6. Release Kinetic Studies[89-95]

For estimation of the kinetic and mechanism of drug release, *invitro* drug release study of nanoparticles were fitted with various kinetic equation like were used to described the release kinetic. The zero order release states that drug release rate was independent of its concentration. The first order release describes, the releases rate from the system was concentration dependent. Higuchi described the releases of drug from insoluble matrix as a square root of time dependent process was based on Fickian diffusion.

1. Zero order - Cumulative % drug release versus time.
2. First order -Log cumulative % drug remaining versus time.
3. Higuchi's model -Cumulative % drug released versus square root of time.
4. Korsmeyer equation / Peppa's model – Log cumulative per cent drug released versus log time.

a. Zero order kinetics:

Zero order release would be predicted by the following equation:

$$At = A_0 - K_0 t$$

Where

At = Drug release at time's'

A_0 = Initial drug concentration.

K_0 = Zero- order rate constant (hr^{-1})

Where the data is plotted as cumulative per cent drug release versus time, if the plot is linear then the data obeys Zero –order kinetics and its slope is equal to Zero order release constant K_0.

First order kinetics :

First order release could be predicted by the following equation:

$$\text{Log } C = \log C_0 - Kt / 2.303$$

Where,

C = Amount of drug remained at time's'

C_0 = Initial amount of drug

K = First order rate constant (hr^{-1})

When the data plotted as log cumulative per cent drug remaining versus time, yield a straight line, indicating that the release follows first order kinetics. The constant 'Kt' can be obtained by multiplying 2.303 with the slope value.

b. Higuchi's model:

Drug release from the matrix devices by diffusion has been described below Higuchi's classical diffusion equation:

$$Q = [D€ / _T (2A - €Cs) Cs t]^{1/2}$$

Where,

Q = Amount of drug release at time't'

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = Solubility of drug in the matrix.

€ = Porosity of the matrix.

$_T$ = Tortuosity.

t = Time (hrs. at which q amount of drug is released.)

Above equation can be simplified assumed that 'D', 'Cs' and 'A' are constants. Then the equation becomes

$Q = Kt^{1/2}$

According to the equation, if cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism.

c. Korsmeyer equation / Peppa's model:

Korsmeyer described a simple relationship to find the mechanism of drug release from polymeric system. To study the mechanism of drug releases from the Nanoparticles formulation, the release data was fitted to the Korsmeyer –Peppa's law equation, which was used to describe the drug release behaviour systems.

$$\mathbf{Mt / M\alpha = Kt^{\eta}}$$

Where,

Mt / Mα = Fraction of drug released at time't'

K = Constant

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides, Log Mt / Mα = Log K + n Log t

In the above equation, "n" value was used to characterize the various release mechanism as mentioned in the table below

Table No: 6. Diffusion exponent and solute release mechanism for cylindrical shape

S.No	Diffusion	Exponent (n) Overall solute diffusion mechanism
1	0.45	Fickian diffusion
2	0.45<n<0.89	Anomalous(non-Fickian) Diffusion
3	0.89	Case - 2transport
4	n>0.89	Super case-2 transport

5.7. Morphology:[96-100]

The nanoparticle morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscoy (SEM) at different magnifications .A few mg of prepared nanoparticles was gold coated using a Hitachi HVSJGB vaccum evaporater. Coated samples were viewed and photographed in a Hitachi S-450 SEM operated at 20kv.

5.8.Anti diabetic activity:

In vitro α- Amylase Inhibition Assay

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 di nitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540nm. Calculation of 50% inhibitory concentration (IC50).The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract .

Experimental Models of *Cassia auriculata* .L

For the study of anti-diabetic an experimental model is selected in such a way that it would satisfy the following:

- The animal should develop hyperglycemia rapidly.
- Pathological changes in the site of induction should result from pancreatitis or damage of β-cells.
- The symptoms should be ameliorated or prevented by a drug treatment effective in human beings.

Treatment Protocol

- Group-I: (Normal control) consist of normal rats given with 10ml/Kg of normal saline, orally.
- Group-II: (Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate through I.P.
- Group-III: Diabetic control received glipizide at a dose of (10mg/Kg orally) for 28 days.
- Group-IV: Diabetic control received Ethanolic extract of *Cassia auriculata* at a dose of (200mg/Kg orally) for 28 days.

- Group-V: Diabetic control received Nano particles of *Cassia auriculata* at a dose of (50mg/Kg orally) for 28 days.

Methodology:

Sample collection:

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocularpuncture.[92] in EDTA coating plasma tubes for the estimation of blood parameters.

6.1. Collection of Flower *Cassia auriculata*.L:

The fresh flower of *Cassia auriculata*.L.was collected in the month of November from vagaikulam,thoothukudi, tamilnadu. The plant was identified and authenticated by The American college, Madurai. The fresh bark was used for the study of macro morphological and microscopy, physicochemical characterization and phytochemical analysis.

6.1.1. Macroscopic characters of Flower *Cassia auriculata*.L :

Organoleptic and macroscopic studies were conducted on in tact and powdered materials of *Cassia auriculata*.L Flower power. Sample was washed, air dried in shade and observed for color, shape, odor, taste, and other surface characteristics. Flowers which were shade dried for 10–15 days.A after drying it was pounded to coarse powder and observed for color, odor and taste.

flowers Characteristics of *Cassia auriculata*.L

- Irregular,
- bisexual,
- bright yellow and large (nearly 5 cm across),
- the pedicels glabrous and 2.5 cm long.
- The racemes are few-flowered, short, erect,
- crowded in axils of upper leaves so as to form a large terminal inflorescence
- The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones.
- The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange.
- The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules

Organoleptic characters:

Table.7 The results of Cassia auriculata. L Organoleptic characters has been tabulated.

COLOUR	Bright yellow
ODOUR	Characteristic
TASTE	Astringent
MARGIN	Crisped

Table No.8. Quantiative Morphology Flower of *Cassia auriculata*.L

LENGTH	2.5-5cm
THICKNES	: 2-3mm
SHAPE	Curved
TEXTURE	Smooth

6.1.2 . Microscopic Characteristics of Flower *Cassia auriculata*.L:

- Flower powder is yellow
- non aromatic,
- Astringent
- The microscopic examination of the powder of *Cassia auriculata*.L Morphological examinations were conducted using a **Binocular zoom** light microscope
- Free handed prepared cross section examined
- stained with freshly prepared dyes safranin and fast green, and

- different grades of alcohol were used to increase visibility.

FIG. 6. Powder Microscopy of Flower of Cassia auricula.L

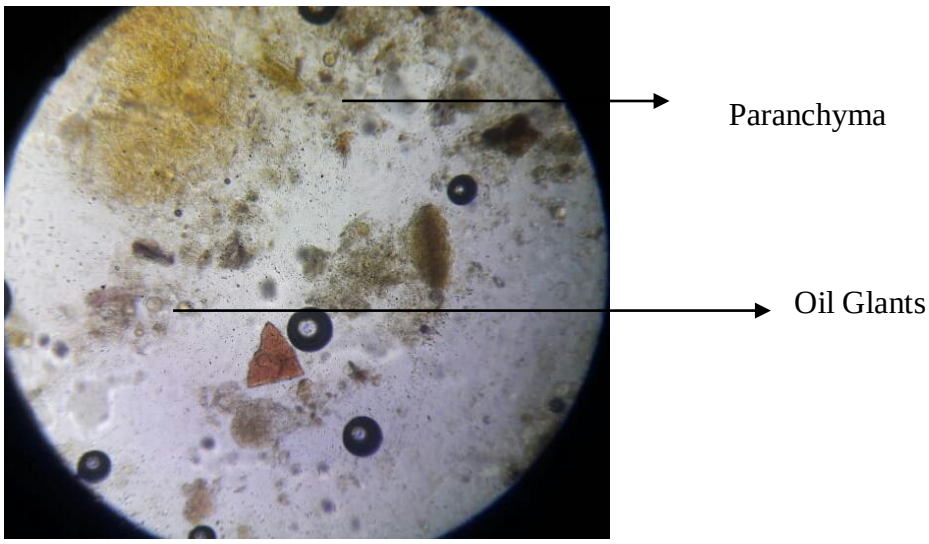


FIG. 7. Powder Microscopy of Flower of Cassia auricula.L

Powder Characterstics Flowe of *Cassia auriculata*.L:

Powder Dark-brown; shows fragments of parenchy Smatous cells, broken unicellular hairs, vessels with spiral thickening, a few prismatic and cluster crystals of calcium oxalate; a few irregular shaped, elongated, lignified, stone cells with narrow lumen in\ singles or groups; fairly large circular to spherical, brown coloured, numerous smooth pollen grains measuring 67-82 µ in dia. having clear exine and intine and a few oil globules

Powder-Dark-brown; shows fragments of parenchy-matous cells, broken unicellular hairs, vessels with spiral thickening, a few prismatic and cluster crystals of calcium oxalate; a few irregular shaped, elongated, lignified, stone cells with narrow lumen in\ singles or groups; fairly large circular to spherical, brown coloured, numerous smooth pollen grains viewed measuring in dia. having clear exine and intine and a few oil globules.

FIG. 8. Pollen grains cells

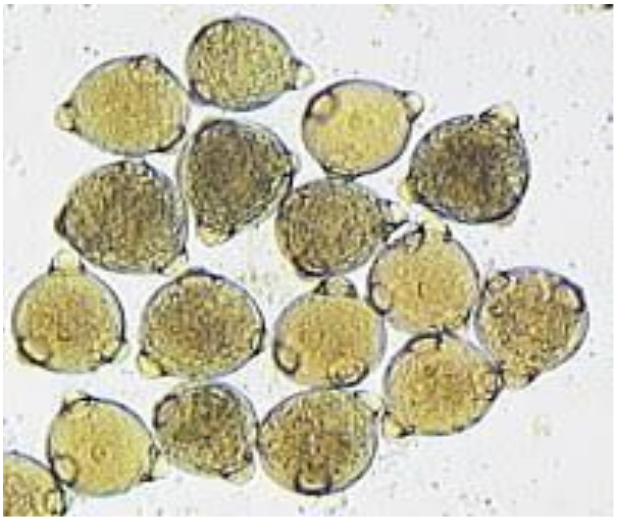


Fig. 9. Stone cells

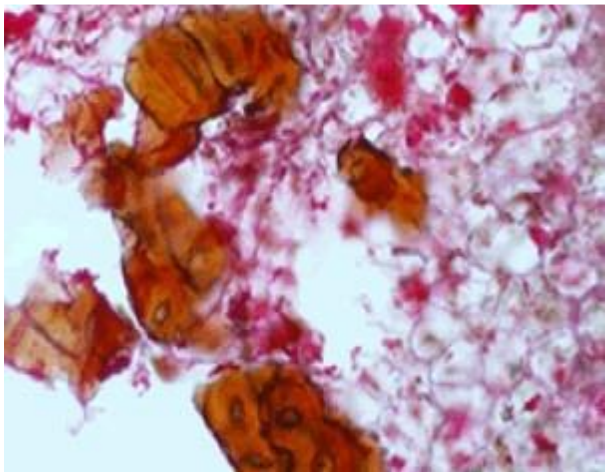
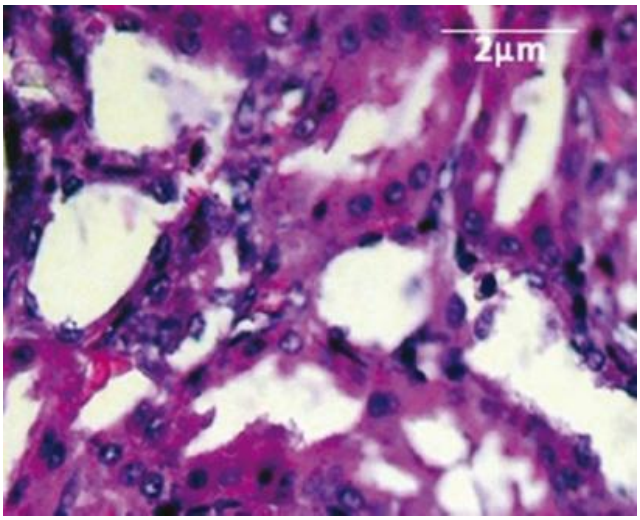


FIG.10. Calcium oxalate cells



Phytochemical and Evaluation

The preliminary Phytochemicals was done in the department and following results obtained. It was noticed that *Cassia auriculata* L. with aqueous mixture has the presence of Carbohydrates, Saponins, Tanins, Proteins, Amino acids and Steroids and pH of was found to be 6, which is mild acidic.

Tests for Alkaloids

1. Mayer's Test: To1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of alkaloids.

Test for Carbohydrates

2. Molisch Test: To 2 ml of the extract, 1 ml of □-naphthol solution and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Tests for Proteins and Amino Acids

3. Lead Acetate Test: To the extract, 1ml of lead acetate solution is added. Formation of a white precipitate indicated the presence of proteins.

Test for Saponins

4. About 1 ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. 1cm layer of foam indicated the presence of saponins.

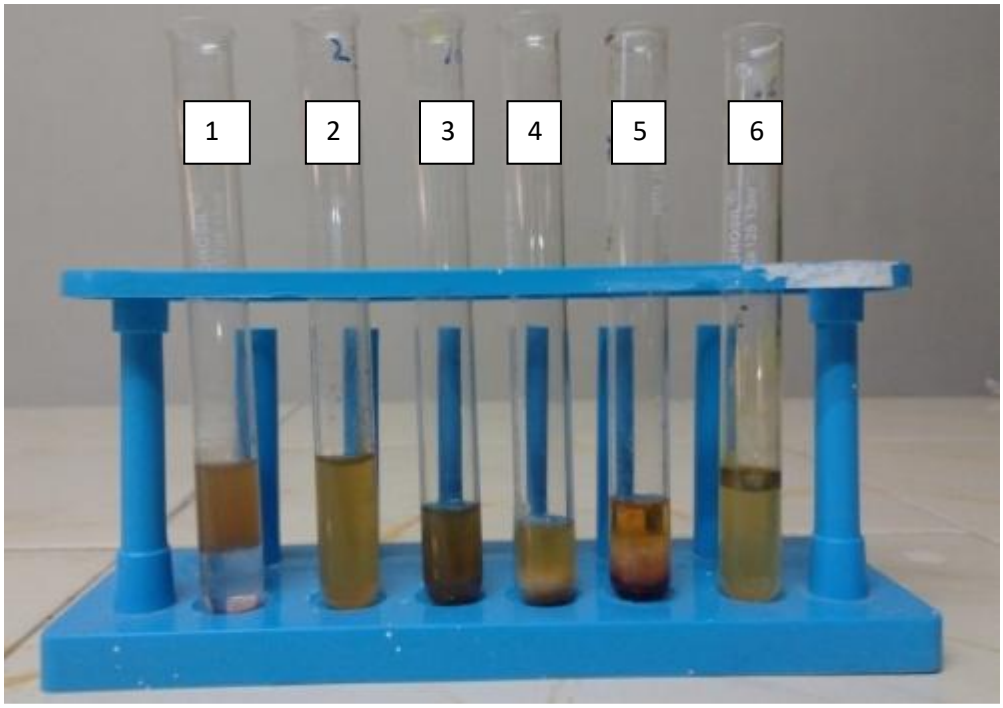
Test for Flavonoids

5. Shinoda Test: To 1 ml of the extract, magnesium turnings were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of red colour showed the presence of flavanoids.

Test for Tannins and Phenolic compounds

6. To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.

FIG.11. Phytochemical and Evaluation



6.1.3. Physicochemical Evaluation Flower of *Cassia auriculata*.L:

Phytochemical screening Phytochemical screening revealed the presence of alkaloids, steroids and tri terpeniods in the chloroform extract, flavonoids and tannins in methanolic extract, phenolic compounds in aqueous extract . The results are shown in Table.7 Physico-chemical parameters for *Cassia auriculata*.L.,

Table -9. Physico-chemical parameters of Cassia auriculata Linn flower

s.no.	Parameter	Determined value % w/w
1	Alcohol soluble extractive value	20
2	Water soluble extractive value	25
3	Pet ether soluble extractive value	1.50
4	Moisture content	12.50
5	Total ash	16
6	Water soluble ash	11.3
7	Acid insoluble ash	11.3
8	Sulphated ash	22.3

Table -10 Physical parameters of Isolated compounds of *Cassia auriculata*.L.flower.

Parameters	Isolated compound
Physical State	Solid
Colour	Yellow
Odour	Astringent
Solubility	Methanol

6.1.4. Phytochemical Investigations Flowe of *Cassia auriculata*.L:

The qualitative chemical tests carried out for the identification of the nature different phytoconstituents present in the powered crude drug. The tests were carried out using standard conventional protocols.

FIG.12. Phytochemical Investigations of *Cassia auriculata*.L Flower

Extraction Process of Flower *Cassia auriculata*.L

Cassia auriculata .L flowers (500g) were extracted with1500 ml of Ethanol by the method of continuous hot extraction at 60 °C for 6 h and evaporated. The residual extract was dissolved in water and used in the study

Table.11. Preliminary phytochemical tests of various successive extracts of *Cassia auriculata*.L., Flower.

NATURE	METHANOL
Alkaloids	+
Flavonoids	+
Phenols	+
Proteins	+
Saponins	+
Steroids	+
Terpenoids	+
Glycosides	+

'+' indicates Presence **'-'** indicates Absence

6.2. Formulation of *Cassia auriculata*.L .Nanoparticles

The entrapment efficiency (%) of the *Cassia auriculata*.L. Loaded nanoparticles formulations (F1 ,F2 ,F3, , and F4) and an optimized F3 was determined and tabulated.

Table No.12 Entrapment efficiency of formulations with drug and copolymer

S.no	Formulation Code	Amount of Drug entrapment(mg)	Percentage Entrapment Efficiency
1	F1	57.86 ±0.45	42.14±0.12
2	F2	36.44±0.24	63.56±0.14
3	F3	6.57±0.19	93.47±0.15
4	F4	10.15±0.32	89.85±0.17

The formulation has showed entrapment efficiency of the formulation F3 showed % entrapment efficiencies of 93.47.The decrease in the % entrapment efficiency then compared to formulation F4 was due to increased copolymer concentration because of higher hydroscopic or steric interactions between the polymer and drug.

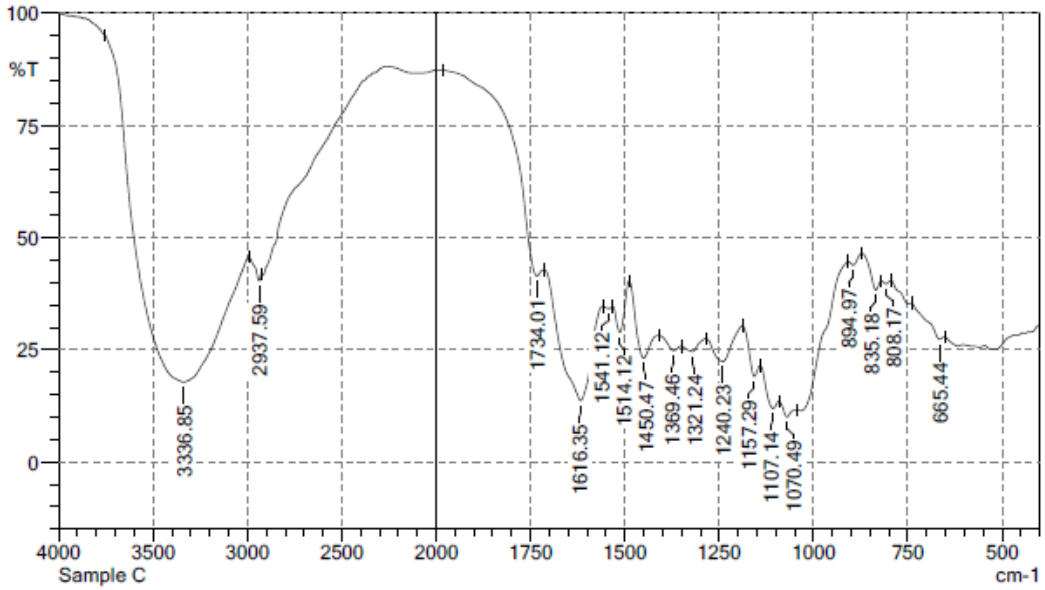
Hence the F3 formulation has shown highest % entrapment efficiency with higher drug loading content when compared with other formulations .So it was selected as an optimized formulation .

6.3. Evaluvation of Cassia auriculata.L NANOPARTICLES:

6.3.1FT-IR Spectrum *Cassia auriculata*. L

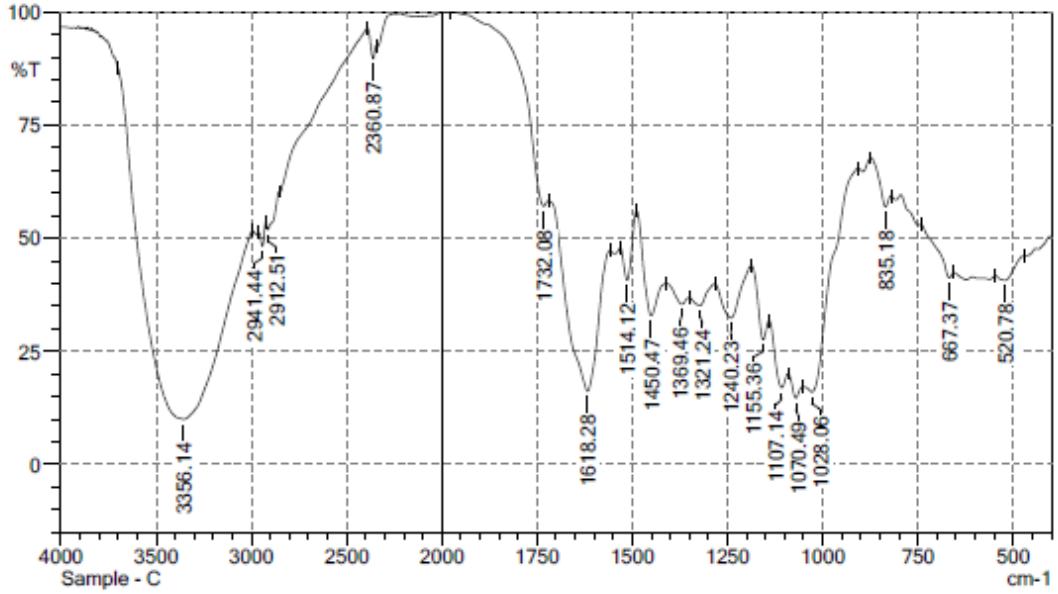
FT-IR Spectrum was taken to study the compatability of the drug and other excipients. Results show that there is no significant interaction of excipients with the drug in the formulation

Fig.13. **FT- IR of *Cassia auriculta.L***



Frequency cm^{-1}	Functional Groups
894.93	C-H
1070.49	C-H
1450.47	O=H
1514.12	N-O
2937.59	C-H
3336.85	C-H

Fig .14. FT-IR of *Cassia auriculata*.L nanoparticle



Frequency cm^{-1}	Groups Assigned
894.97	C-H
1070.49	C-O
1450.47	O-H
1514.12	N-O
1562.06	N-O
2937.59	C-H
3336.85	C-H

Important peaks, seen in the FT-IR spectrum of extraction of Cassia auriculata.L are exhibited in the nanoparticle of Cassia auriculata.L formulation. Hence there was no significant interaction between the drug and the excipients.

6.4. *In vitro* Drug release profile of *Cassia auriculata*.L. Nanoparticles

The in vitro drug release studies were performed for the the prepared for the prepared Nanoparticles formulations (F1, F2, F3, and F4). Results were tabulated and graphs were made by plotting cumulative % drug release against time in hours on the y axis and x axis respectively. The *in vitro* drug release characteristics for prepared Nanopariticle formulations were evaluated with the help of release profiles in graphical plots.

The formulation F3 has showed a drug release of in 93.47 % in 24 hours. Where as the F4 Showed a drug release of 89.85 %.The decrease in drug release when compared with the F3 formulation was found to be due increased hydrophobic or steric interactions between drug and polymer. Hence the F3 formulation was selected as the best formulation with highest percent drug release and also having the higher % encapsulation effienency then compared to the other formulations.

Table No: 13. *In vitro* drug release data of F1 formulation

S.NO	Time in hrs	Percentage Drug release	Cumulative % drug Release
1.	1	25.30	25.32
2.	2	48.73	48.76
3.	3	69.90	69.95
4.	4	91.43	91.43
5.	5	98.14	98.14

Fig: 15. *In vitro* drug release data of F1 formulation

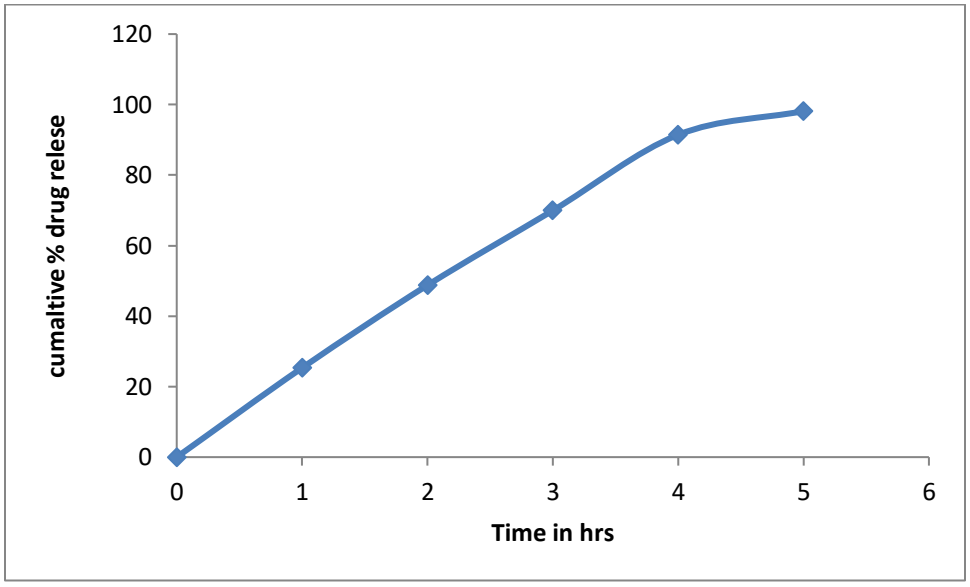


Table No: 14 *In vitro* drug release data of F2 formulation

S.No	Time in hours	Percentage Drug release	Cumulative % drug release
1	1	10.12	10.12
2	2	22.45	22.45
3	3	35.49	35.49
4	4	45.19	45.19
5	5	65.91	65.91
6	6	75.75	75.75
7	7	80.5	80.5
8	8	91.45	91.45
9	9	98.41	98.41

Fig: 16. In vitro drug release data of F2 formulation

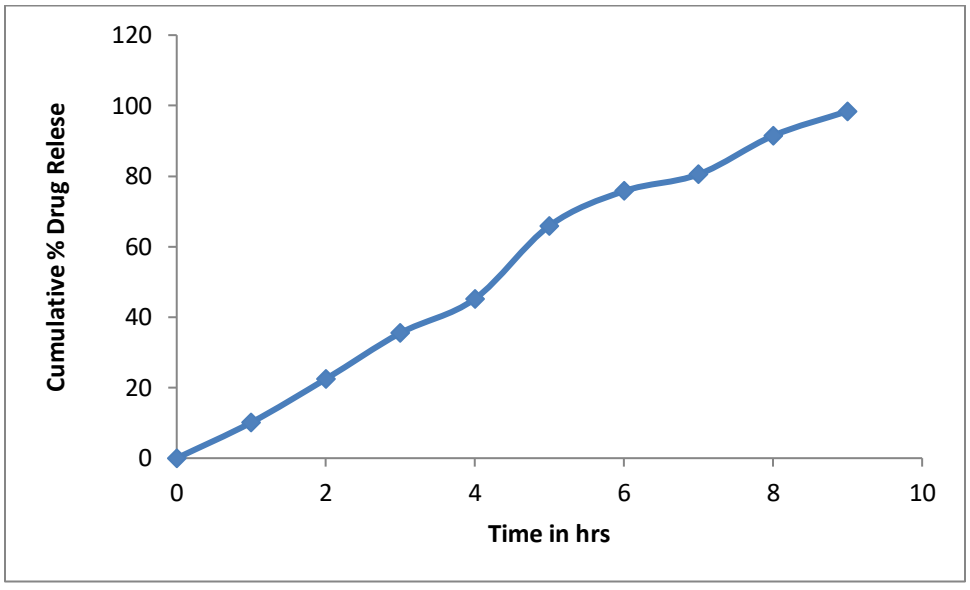


Table No: 15. *In vitro* drug release data of F3 formulation

S.No	Time in hrs	Percentage Drug release	Cumulative % drug release
1	1	3.8	3.8
2	2	7.1	7.1
3	3	13.1	13.1
4	4	18.5	18.5
5	5	23.9	23.9
6	6	28.9	28.9
7	7	31.92	31.92
8	8	36.12	36.12
9	9	40.19	40.19
10	10	43.9	43.9
11	11	47.5	47.5
12	12	50.75	50.75
13	13	54.42	54.42
14	14	57.92	57.92
15	15	60.95	60.95
16	16	65.01	65.01
17	17	69.95	69.95
18	18	73.75	73.75
19	19	78.12	78.12
20	20	81.95	81.95
21	21	86.25	86.25
22	22	91.17	91.17
23	23	94.11	94.11
24	24	98.98	98.98

Fig: 17. *In vitro* drug release data of F3 formulation

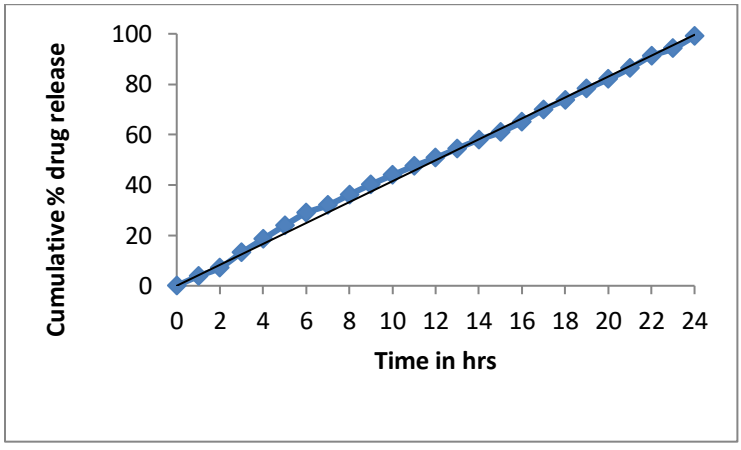
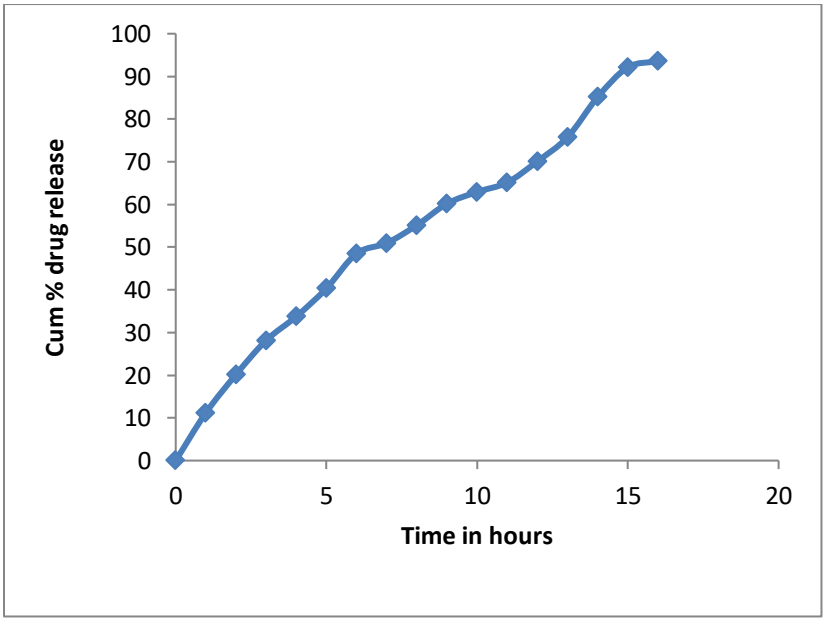


Table No: 16. *In vitro* drug release data of F4 formulation

S.No	Time in hrs	Percentage Drug release	Cumulative % drug release
1	1	11.19	11.19
2	2	20.15	20.15
3	3	28.15	28.15
4	4	33.75	33.75
5	5	40.35	40.35
6	6	48.5	48.5
7	7	50.95	50.95
8	8	55.15	55.15
9	9	60.18	60.18
10	10	62.93	62.93
11	11	65.15	65.15
12	12	70.12	70.12
13	13	75.76	75.76
14	14	85.18	85.18
15	15	92.12	92.12
16	16	93.59	93.59

Fig : 18. In vitro drug release data of F4 formulation



6.5. Stability study

The optimized formulation F3 was subjected to stability study for one month at 4 ^{o}C (refrigerator) room temperature and at 45 ^{o}C / 75% RH. At the interval of 30 days samples of nanoparticles formulation were taken and evaluated for the entrapment efficiency and *in vitro* release of drug (Table 17 to 19). Entrapment efficiency of optimized formulation F9 kept at 9^{o} C shows a release rate of 95.5% after 30 days of stability study .The entrapment efficiency of formulation got decreased on exposure to higher temperature. The percentage entrapment of formulation kept on room temperature and at 45^{o}C /75% RH were 82% 81% reapectively after 30 days of stability study (Table 14)

The in vitro release data of optimized formulation f 9 shows that the nanoparticles formulations are more stable at 45 ^{o}C / 75% RH (stability chamber), .The Nanoparticles formulation kept at 4 ^{o}C showed a cumulative release of 89.89% after 30 days of stability studies (Table 17 to 19)

Table No: 17. Stability Study Data For F3 Formulation 4° C / 75% RH

Time in hrs	Cumulative % Drug release		
	After One month	After Two months	After Three months
1	8.2	7.9	6.9
2	11.25	10.69	10.01
3	13.95	12.68	11.23
4	18.41	17.25	16.25
5	23.92	22.81	20.67
6	27.67	25.65	23.24
7	30.52	29.28	28.78
8	34.52	32.5	30.79
9	40.29	39.48	37.48
10	45.64	43.23	41.43
11	48.38	47.38	45.41
12	51.96	50.92	49.41
13	54.29	53.29	51.25
14	59.29	58.23	57.21
15	62.84	61.25	60.32
16	69.79	68.29	67.21
17	71.82	70.21	70.01
18	73.29	72.23	71.23
19	79.84	78.84	76.24
20	86.49	85.49	83.49
21	89.63	87.63	85.25
22	92.09	91.68	91.02
23	95.38	94.25	94.01
24	98.95	97.12	96.19

Fig : 19. Stability Study Data For F3 Formulation 4 °C / 75% RH

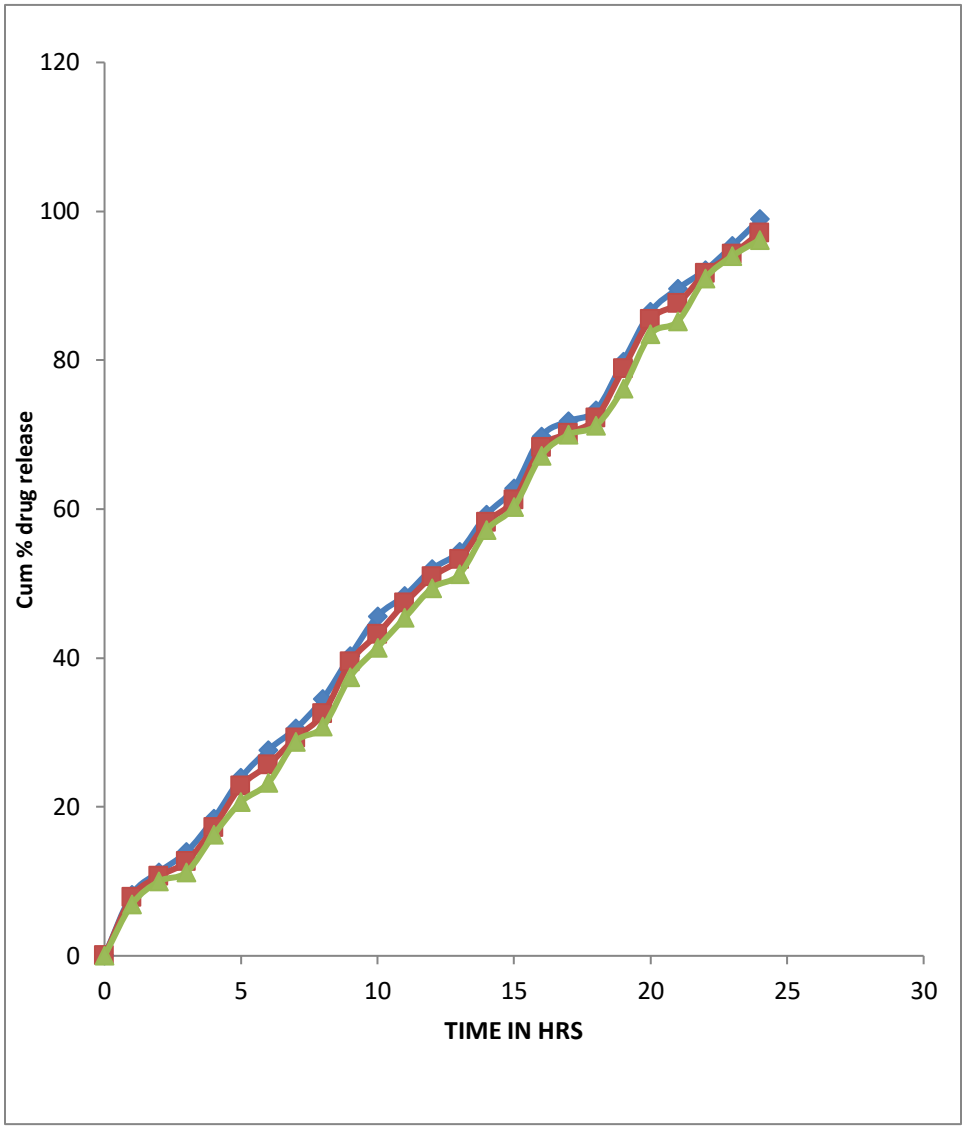


Table No: 18. Stability Study Data For F3 Formulation Room temperature

Time in hrs	Cumulative % Drug release		
	Affter One month	Affter Two month	Affter Three month
1	8.2	7.9	6.9
2	11.75	10.12	7.92
3	14.79	13.14	10.58
4	18.92	15.42	13.12
5	23.95	22.5	20.24
6	27.69	25.86	23.45
7	31.72	29.42	27.49
8	35.99	34.42	33.42
9	40.92	39.42	37.42
10	44.65	42.51	40.12
11	47.42	45.12	43.12
12	51.99	49.12	47.12
13	54.79	52.81	51.02
14	59.29	56.81	53.81
15	63.84	62.19	60.19
16	69.29	67.12	63.12
17	72.95	70.58	68.58
18	79.84	76.24	73.24
19	84.45	82.14	80.12
20	89.23	85.14	83.14
21	92.08	91.23	90.14
22	94.95	93.01	92.65
23	95.56	94.24	93.23
24	98.45	96.47	94.23

Fig : 20. Stability Study Data For F3 Formulation Room temperature

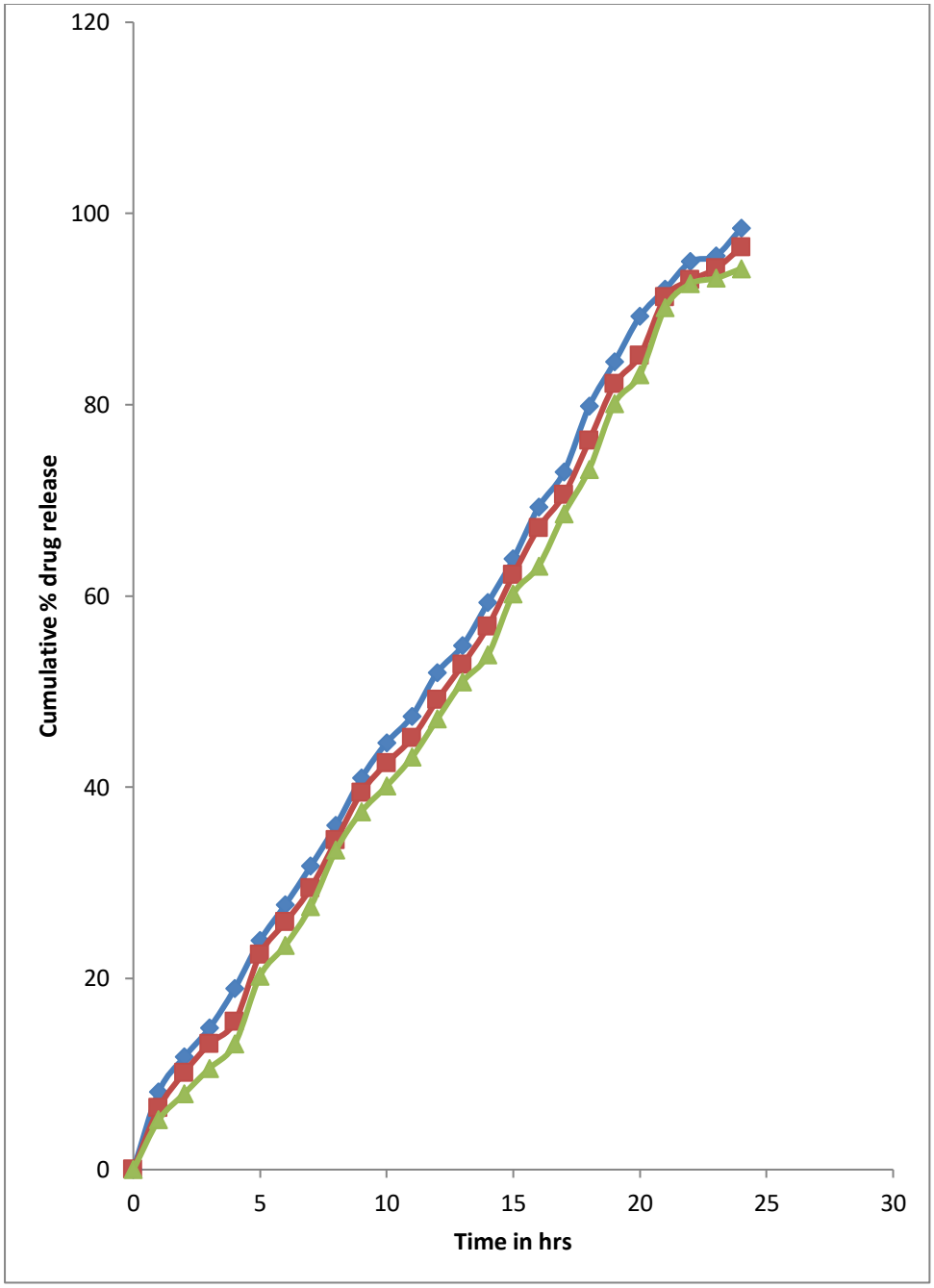
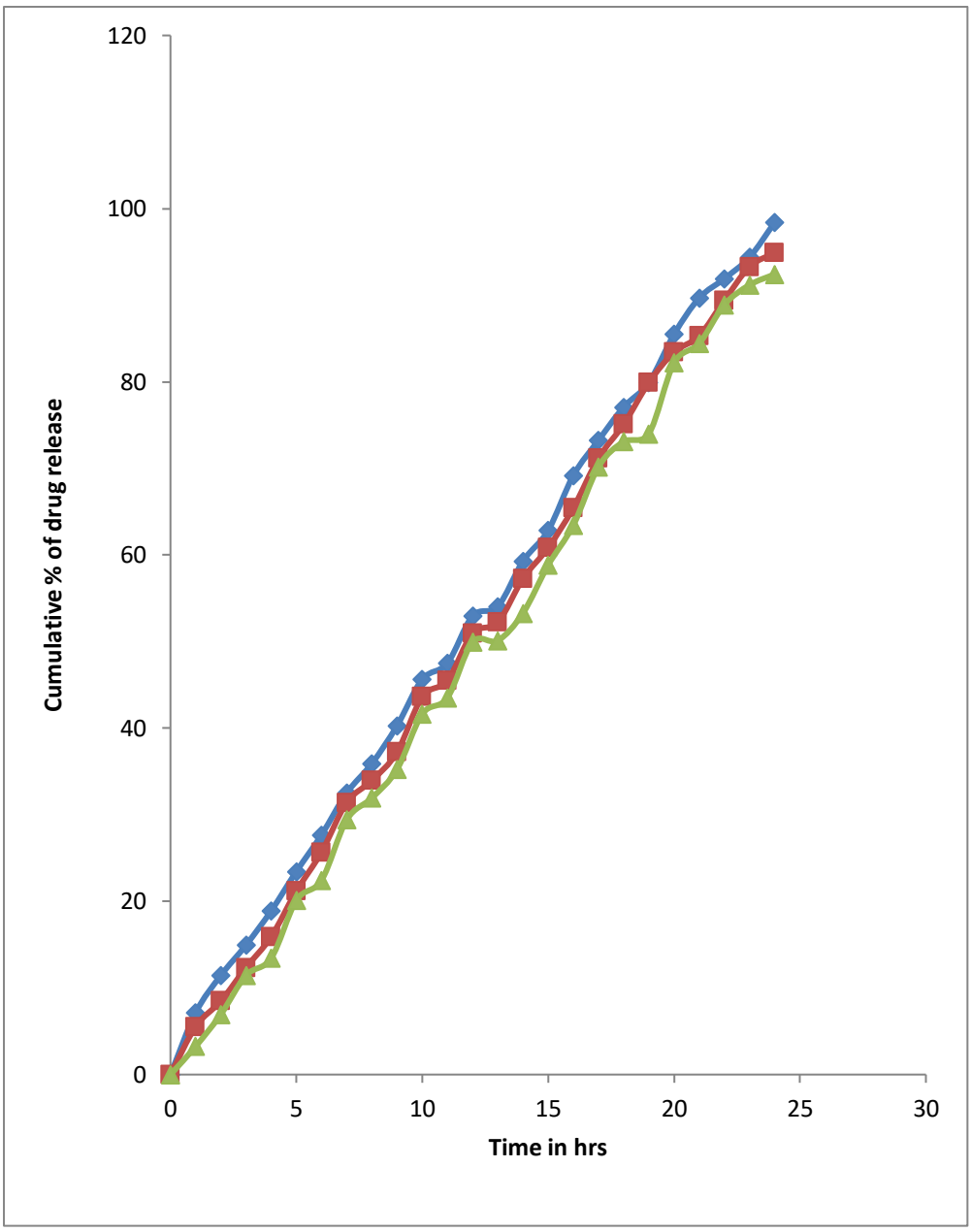


Table No: 19. Stability Study Data For F3 Formulation F3 AT 4^0 C RH

Time in hrs	Cumulative % Drug release		
	Affter One month	Affter Two months	Affter Three months
1	8.2	7.9	6.9
2	11.45	8.49	6.95
3	14.95	12.35	11.45
4	18.91	15.92	13.45
5	23.45	21.19	20.12
6	27.69	25.69	22.45
7	32.52	31.42	29.42
8	35.92	33.95	31.95
9	40.29	37.25	35.25
10	45.64	43.63	41.64
11	47.52	45.52	43.52
12	52.96	50.96	49.96
13	54.07	52.23	50.12
14	59.29	57.29	53.29
15	62.84	60.84	58.84
16	69.19	65.45	63.45
17	73.25	71.2	70.2
18	77.09	75.09	73.09
19	79.92	79.92	74.01
20	85.49	83.42	82.19
21	89.69	85.32	84.45
22	91.92	89.42	88.91
23	94.39	93.29	91.19
24	98.43	94.92	92.43

Fig : 21. Stability Study Data For F3 Formulation F3 AT 4^0 C RH

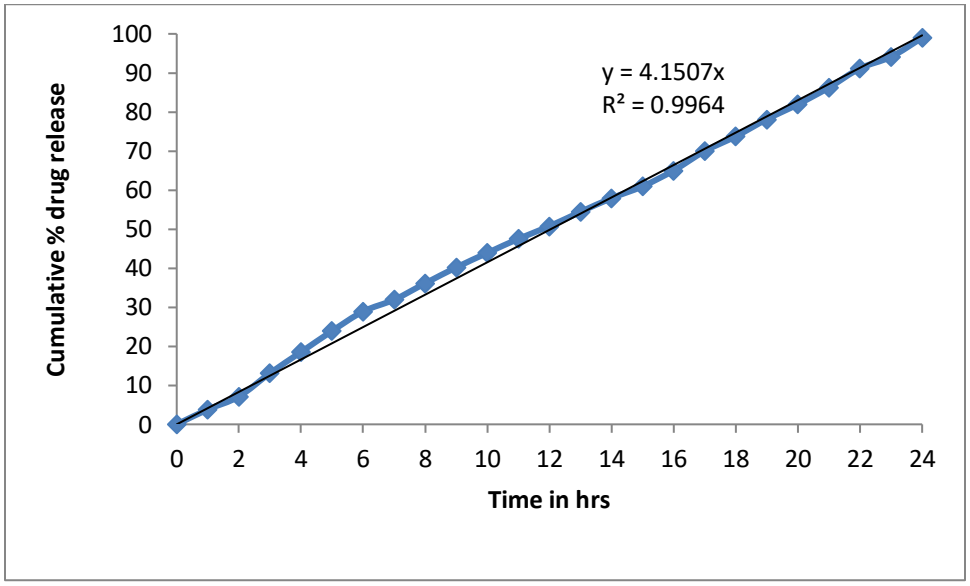


6.6. Release Kinetics Studies

The release kinetics was studied for an optimized formulation F3 by plotting the graphs for different kinetic models by using the in vitro drug release data

a) Zero Order Plot:

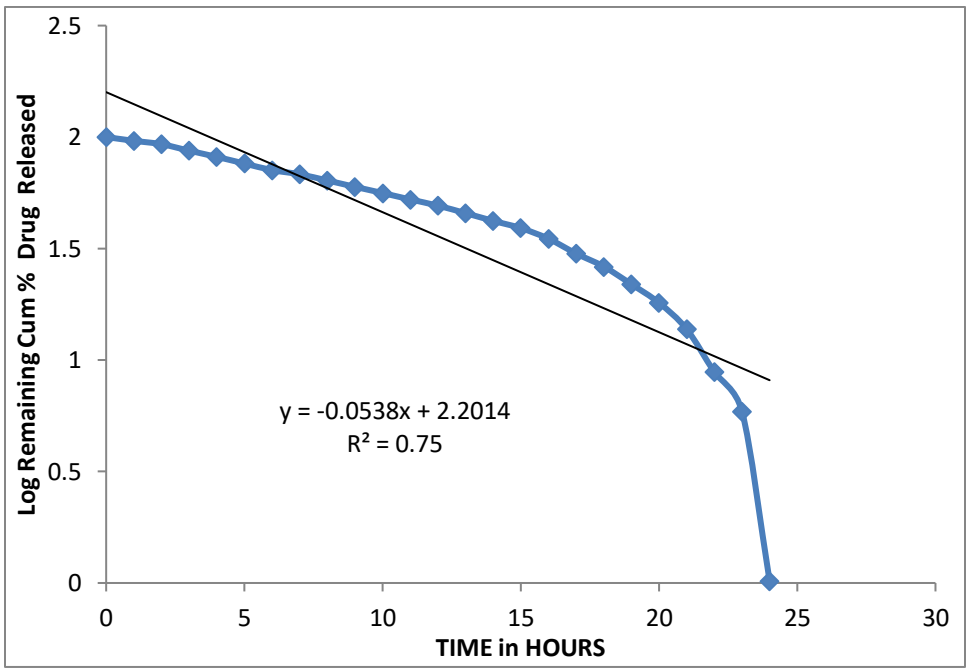
Fig No : 22. Zero –order release profile for an optimized F3 formulation



Zero order release kinetics	
R_2	0.996

b) First Order Plot:

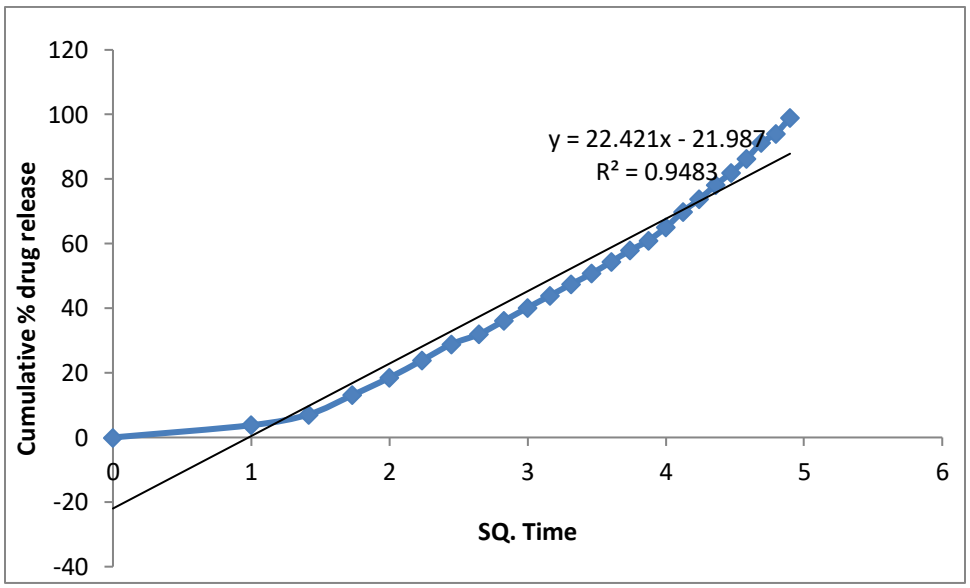
Fig: 23. First Order Release Profile For an Optimized F 3 Formulation



First order release kinetics	
R_2	0.75

C)Higuchi Plot:

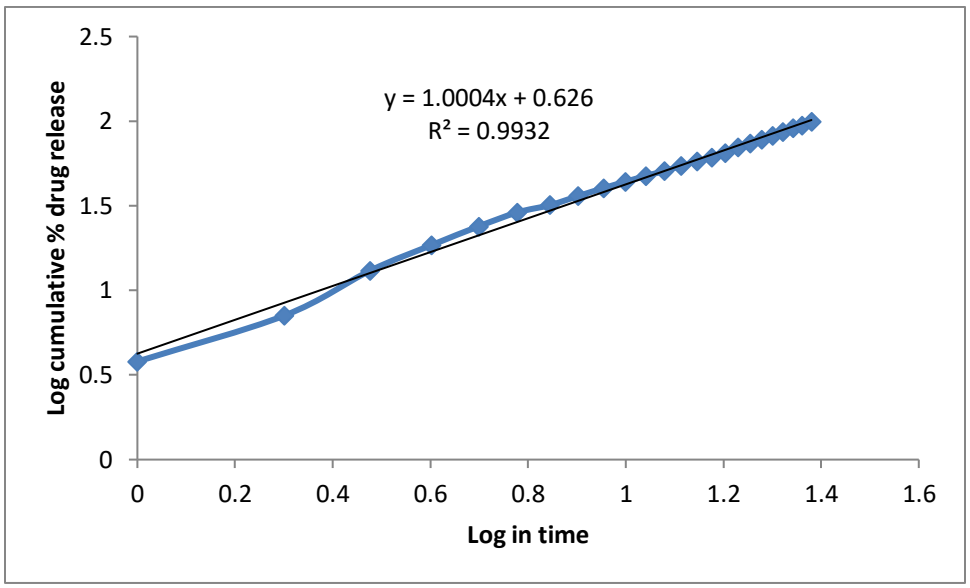
Fig: 24. First Order Release Profile For an Optimized F3 Formulation



Higuchi Plot release kinetics	
R_2	0.948

d) Korsmeyar Plot:

Fig: 25. First Order Release Profile For an Optimized F3 Formulation



Korsemeyar Plot release kinetics	
n Value	0.626

Zero order plot:

Zero order plot of a freeze dried formulation F3 was fond to be linear with a regression value of, 0.996 which signifies that the drug was released in a controlled manner form the nanoparticles during the release study.

First order plot:

The first order plot was made by plotting log remaining cumulative % drug release against time and the regression value was found to be 0.75 which indicates that drug release was not followed the first order rate kinetics.

Higuchi plot:

Higuchi plot was found to be of linear with a regression value of ,0.948 which indicates that diffusion was one of the mechanisms of the drug release from Nanoparticles matrices.

Korsmeyer - Peppas plot:

The type of *invitro* mechanisam of drug release was best explained by Korsmeyer – Peppas plot. The plot was found to be liner with R^2 value

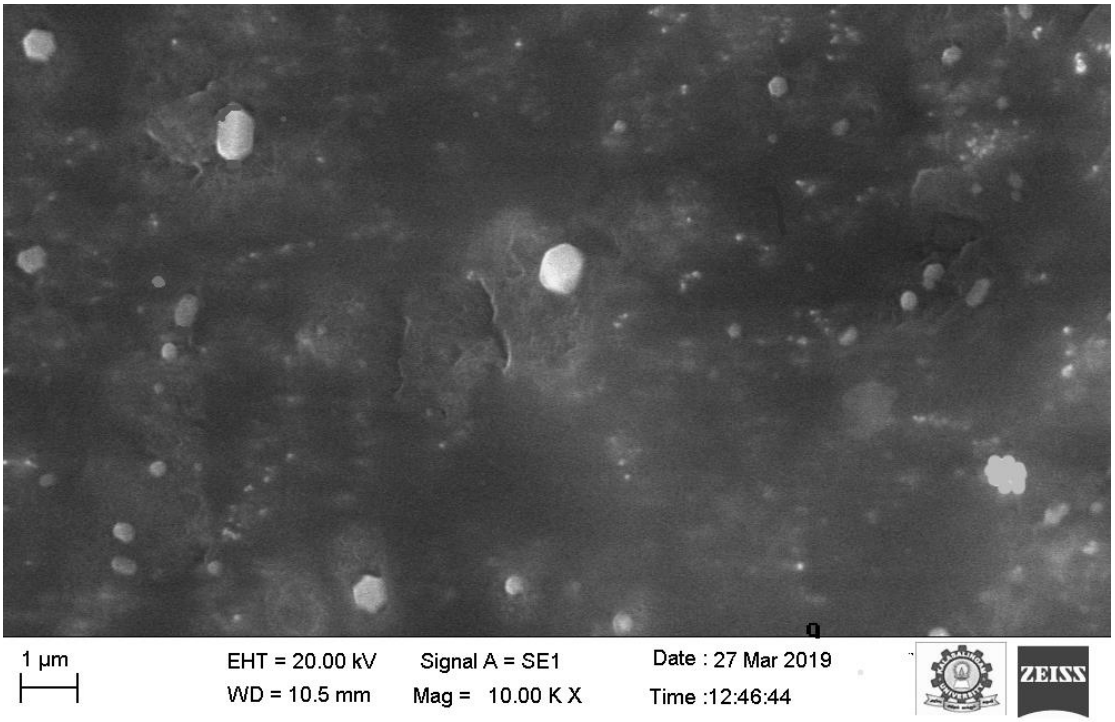
And diffusion exponent n value was 0.626, According to Korsmeyer – Peppas equation, mechanisam of drug release based on (given in table no:4), which indicates that mechanism of drug release from copolymer matrices was followed
Anomalous (non-Fickian) diffusion. So the kinetic of an optimized F3 formulation with different kinetic models has showed that the *Cassia auriculata*.L . Release mechanism was found to be Anomalous (non-Fickian) based on n value from Korsmeyer –Peppas plot.

6.7. Morphology:

The nanoparticles morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscopy (SEM) at different magnifications. A few mg of prepared nanoparticles was gold coated using a Hitachi HVSJGB vaccum evaporter. At 20 kv. It was found that the nanoparticles were mostly spherical in shape

and some nanoparticles are slightly elongated, discrete and almost uniform in size . The morphology on the nanoparticles is shown in Fig: 24

Fig No: 26. SEM Photograph



Morphology:

SEM photograph of the prepared nanoparticles reveal that they are discrete, spherical in shape, amorphous in nature and had a matrix type structure. No drug crystals have been identified.

6.8. Antidiabetic activity:

In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered. Persistent hyperglycemia is a major contributor to such metabolic alterations, which lead to the pathogenesis of diabetic complications. The study was designed by L Pari, M Latha in 2002, to study the effect of *Cassia auriculata* flower extract on hepatic glycolytic and gluconeogenic enzymes and STZ-diabetic rats were given the plant's extract per os for 30 days. In conclusion, the observations showed that the aqueous

extract possessed an antihyperglycemic effect and suggested that enhanced gluconeogenesis during diabetes is shifted towards normal and that the extract enhanced the utilization of glucose through increased glycolysis. The effect of the extract was more prominent than that of glibenclamide

Estimation of blood glucose:

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method. [93]

Table no: 20 illustrates the levels of initial and final blood glucose, and change in body weight, in normal rat, and treatment control animals in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and 50mg/kg was increased the body weight non-significantly as compared to normal control animals.

Fasting blood glucose level was significantly increased 219.48 ± 6.96 in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range in diabetic rats treated with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg).

Estimation of blood glucose:

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method. [93]

Table No: 20. Effect of Ethanolic extract of *Cassia auriculata* on initial and final body weight and blood glucose in normal and treated animals.

GROUP	Body weight (g)		Blood glucose (mg / 100ml) Initial	Blood glucose (mg / 100ml) Final
	Initial	Final		
G1	234 ± 7.25	242± 7.60	92.60 ± 3.34	92.75 ± 3.80
G2	236 ± 7.48	176 ± 4.46**(a)	90.94 ± 3.30	222.75 ± 6.90**(a)
G3	238 ± 7.52	240 ± 7.55	88.95 ± 3.22	123.35 ± 4.30**(b)
G4	232± 7.18	242 ± 7.45	84.25± 3.16	141.50± 5.32**(b)
G5	230 ± 7.10	244 ± 7.50	94.36 ± 3.75	131.15 ± 4.40**(b)

- Values are expressed as mean **±** SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table no: 20. Illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control animals in each group.

The levels of total hemoglobin, and plasma insulin levels were decreased significantly where as glycosylated heamoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin, returned to near normal range in diabetic rats treated with Ethanolic

extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg).

Plasma insulin:

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit[94] with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of total haemoglobin and glycosylated haemoglobin:

Total haemoglobin was determined by the method of Drabkin and Austin (1932) [5] and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman (1981). [96]

Table no: 21.E ffect of Ethanolic extract of *Cassia auriculata* on plasma insulin, Hemoglobin & Glycosylated hemoglobin in normal and treated animals.

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA_1 (%)	Plasma Insulin (µU/ml)
G1	12.90 ± 1.68	0.47 ± 0.06	40.58 ± 2.85
G2	6.34 ± 0.82**(a)	0.96 ± 0.16**(a)	13.85 ± 1.90**(a)
G3	14.4 ± 1.48**(b)	0.42 ± 0.07**(b)	29.48 ± 2.52**(b)
G4	12.79 ± 0.90**(b)	0.49 ± 0.14**(b)	26.70 ±2.50**(b)
G5	11.98 ± 1.22**(b)	0.44± 0.05**(b)	28.90± 2.80**(b)

- Values are expressed as mean **±** SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table no: 21. shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL),Low density lipoprotein(LDL) and phospholipids of normal and experimental animals in each group.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein(LDL) and phospholipids levels were significantly increased, where as HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) for 28 days resulted in marked decrease in total cholesterol, triglycerides, Low density lipoprotein(LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

Estimation of lipid & lipoprotein:

Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970) (total cholesterol), [97] Gidez and Webb (1950) (HDL-cholesterol), [98] Zilversmith and Davis (1950) (phospholipids) [99] and Rice (1970) (triglycerides). [100]

Table no: 21. Shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein(LDL) and phospholipids of normal and experimental animals in each group.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein(LDL) and phospholipids levels were significantly increased, where as HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) for 28 days resulted in marked decrease in total cholesterol, triglycerides, Low density lipoprotein(LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

Table No.22. Serum lipids of Normal and experimental groups.

GROUPS	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)
G1	88.30 ± 2.62	93.75 ± 2.68	61.65 ± 1.70	131.80 ± 2.42	16.50 ± 1.52
G2	231.35 ± 6.72**(a)	163.60 ± 4.62**(a)	31.72 ± 1.40**(a)	222.60 ± 6.45**(a)	38.65 ± 2.45**(a)
G3	123.84 ± 3.40**(b)	99.65 ± 2.54**(b)	48.90 ± 1.43	155.48 ± 3.80	28.05 ± 1.89**(b)
G4	133.58 ± 3.70**(b)	120.75 ± 2.88**(b)	39.40 ± 1.37**(b)	163.70 ± 4.18**(b)	29.24 ± 1.83**(b)
G5	123.20 ± 3.40**(b)	96.80 ± 2.64**(b)	43.70 ± 1.64**(b)	160.45 ± 3.85**(b)	24.44 ± 1.78**(b)

- Values are expressed as mean **±** SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Alloxan causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. The mechanism of alloxan action was fully described elsewhere (Lazarow, 1964; Colca et al., 1983).[111,112] In our study, we have observed a significant increase in the plasma insulin level when alloxan induced diabetic rats were treated with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) this could be due to potentiation of the

insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens (Alberti and Press, 1982). [113] Glycosylated haemoglobin (HbA_1C) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) [114] and the amount of increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979).[115] During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan induced diabetic rats (Sheela and Augusti, 1992).[116] Administration of Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) for 28 days prevents a significant elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg).

The body weight was decreased in alloxan diabetic rats. Administration of Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and 50mg/kg increases the body weight in alloxan induced diabetic rats. The ability of Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

The level of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coronary heart disease (CHD). [117] Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. [18] The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low density lipoprotein (LDL) levels were brought to near normal by the treatment with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg)in alloxan induced diabetic rats.

DISSCUSION

The effect of Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and 50mg/kg on diabetic hyper triglyceridemia could be through its control of hyperglycaemia. This is in agreement with the facts that:

1. The level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations.[119]
2. Improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides. [120]

The main 'anti-atherogenic' lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver [121] and thereby it acts as a protective factor against coronary heart disease (CHD). [122]

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats. [123] Our results clearly show that the level of HDL-cholesterol was increased in alloxan induced diabetic rats when treated with Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg). These results suggest that Ethanolic extract of *Cassia auriculata* and nano particles of *cassia auriculata* at a dose of 200 and (50mg/kg) has protective effect against alloxan-induced diabetes and its complications

CONCLUSION

Medicinal plant species constitute important alternatives to conventional medicine in a large number of developing countries, especially within poor communities that inhabit rural areas and lack access to health services. *Cassia auriculata.*L flowers might be a potential alternative agent for antidiabetic activity. Hence it is anticipated that *Cassia auriculata* flower would be a useful pharmaceutical material to treat diseases. This investigation may focus research field to develop clinical studies which might be of great scientific contribution for the society. The importance of medicinal plants in traditional health care practices clues to new areas of research and in biodiversity conservation.
The two *invitro* antidiabetic methods have been performed and found to be α-amylase inhibition and glucose uptake of different extracts. To identify the active constituents. Further studies are required to purify the active principle and study the molecular mechanism of the exact pathway. This information's will be useful for the development of alternative method rather than insulin and hypoglycemic agents for the treatment of diabetes mellitus.

From the results of the present experiments it may be concluded that formulation F3 showing formulation of Nanoparticle size, high percentage of entrapment and desired sustained release of Nanoparticle. Hence F3 formulation was optimized one.

The optimized formulation F3 was found to follow zero order release pattern which was revealed by the linearity shown from the plot of time versus concentration.

Nanoparticle formation were stable for 4 weeks at 4^0C and affirm the drug leakage – increased the higher temperature. Comparative study of Nanoparticle formulation with the marketed. The Nanoparticle formulation revealed that anti diabetic activity was released from the Nanoparticle formulation in a sustained manner for 24 Hours.

Thus the aim of the project was achieved by optimizing the formulation Parameters.

SUGGESTION FOR FUTURE STUDIES

As an extension of this work, the following area are planned to do in future to develop a suitable candidate for nanoparticle drug delivery system.

1. Isolation and purification of active constituents
2. Bioavailability studies.
3. Pharmacokinetics studies.

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