DEVELOPMENT OF ANTIMICROBIAL ACTIVITY OF *Senna alata Linn* NIOSOMAL GEL

A Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI-600 032

In partial fulfilment of the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

C.PONMUDI

Reg No: 261710102

Under the guidance of

A.ABIRAMI, M.pharm.,(Ph.D.).,

Asst. Professor

DEPARTMENT OF PHARMACEUTICS

K.M.COLLEGE OF PHARMACY

UTHANGUDI, MADURAI-625107

MAY-2019

CERTIFICATE

This is to certify that the dissertation entitled “DEVELOPMENT OF ANTIMICROBIAL ACTIVITY OF *Senna alata Linn* NIOSOMAL GEL submitted by Mrs.C.PONMUDI (Reg. No: 261710102) in partial fulfilment 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 under 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 universities.

GUIDE

Mrs.ABIRAMI. M.pharm., (Ph.D)

Asst.Professor,

Department of Pharmaceutics,

K.M.college of pharmacy,

Uthangudi, Madurai-625107

H.O.D

Dr.S.MOHAMED HALITH, M.pharm. Ph.D

Professor & Head,

Department of Pharmaceutics,

K.M.college of pharmacy,

Uthangudi, Madurai-625107

PRINCIPAL

Dr.M.SUNDARAPANDIAN, M.pharm, Ph.D.,

Principal,

K.M.college of pharmacy,

Uthangudi, Madurai-625 107

CERTIFICATE

This is to certify that the dissertation entitled “DEVELOPMENT OFANTIMICROBIAL ACTIVITY OF *Senna alata Linn* NIOSOMAL GEL submitted by Mrs.C.PONMUDI (Reg. No: 261710102) K.M.COLLEGE OF PHARMACY, Madurai-625107 in partial fulfilment of the university rules and regulation for the award of MASTER OF PHARMACY IN PHARMACEUTICS under 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 universities.

GUIDE

Mrs.ABIRAMI. M.pharm., (Ph.D)

Asst.Professor,

Department of Pharmaceutics,

K.M.college of pharmacy,

Uthangudi, Madurai-625107

H.O.D

Dr.S.MOHAMED HALITH, M.pharm. Ph.D

Professor & Head,

Department of Pharmaceutics,

K.M.college of pharmacy,

Uthangudi, Madurai-625107

PRINCIPAL

Dr.M.SUNDARAPANDIAN, M.pharm, Ph.D

Principal,

K.M.college of pharmacy,

Uthangudi, Madurai-625 107

ACKNOWLEDGEMENT

AN EXPRESSION OF GRATITUDE

“Praise be to god

The secret of success is undaunted order, motivation, dedication, confidence on self and above the blessing of god. I bow in reverence to almighty for bestowing upon me all kindness that has helped me throughout the journey of my life. The key to joy and true success is found in blessed thankfulness hereby take this opportunity to acknowledge all those who have helped me in the completion of this dissertation work.

Let me first thank ***almighty*** *for giving me life and my* ***Parents*** *for educating me and keeping my requirement in priority at all situations. Without their unconditional support and encouragement it would have been impossible to pursue my interest.*

I express my heartfelt thanks to honourable Chairman ***Prof.M.Nagarajan, M. Pharm., M.B.A., DMS (I.M), DMS (B.M),*** *K.M College of pharmacy, Madurai for his encouragement and providing qualified staffs to complete my thesis work in such a calibre.*

I am greatly indebted to thank ***Dr.M.Sundarapandian, M.Pharm, Ph.D*** *Principal, K.M College of pharmacy, Madurai for his support and constant encouragement during my course of study.*

It is a genuine pleasure to express my deep sense of thanks and gratitude to my mentor, philosopher and guide ***Prof.Dr.S.MOHAMED HALITH., M.Pharm. Ph.D*** *Professor& Head Dept of Pharmaceutics K.M College of pharmacy, Madurai. His dedication and keen interest above all his overwhelming attitude to help his student had been solely and mainly responsible for completing my work. His timely advice meticulous scrutiny advice and scientific approach have helped me to very great to accomplish this task.*

It is a genuine pleasure to express my deep sense of thanks and gratitude to my guide ***Mrs.Abirami M.Pharm. (Ph.D)*** *Asst. Professor, Department of Pharmaceutics K.M College of pharmacy, Madurai. Her dedication and keen interest above all his overwhelming attitude to help his student had been solely and mainly responsible for completing my work.*

I express my sincere thanks to ***Dr.Hariharan****,* ***and Mrs.Sathyapriya Asst.Professor,*** *Department of Pharmaceutics K.M College of pharmacy, Madurai for their help and support during my work.*

I wish to express a special thanks to ***Prof.M.Prakash*** *for the support to complete this work successfully in time.*

I express my sincere thanks to ***Mrs.Shanthi, Mrs.Aruna and Mrs.Priyadharshini Asst. Professor,*** *Department of Pharmaceutics K.M College of pharmacy, Madurai for their help and support during my work.*

*It is my duty to say a special word of thanks to Mrs****.Shanthi B.A., M.L.I.S.c. M.Phil., Librarian Mrs.Lathakalayanasundari*** *for their timely help during this work. A Special work of thanks to* ***all the professor and Assistant professor*** *of all departments for their kind holly hortatory constant encouragement and expertise during this course.*

I wish to express a special thanks to ***Mrs.Vellamal and Mrs.Jeyanthi*** *lab Assistant for their constant valuable support.*

I express my deep sense gratitude to my beloved friends ***Mrs.Sathiya priya Mrs.Annal thamaraiselvi,*** *for their constant valuable support.*

I wish to express a very special thanks to ***Mr.A.Elangovan*** *my* ***husband*** *to encouragement and all time support for me.*

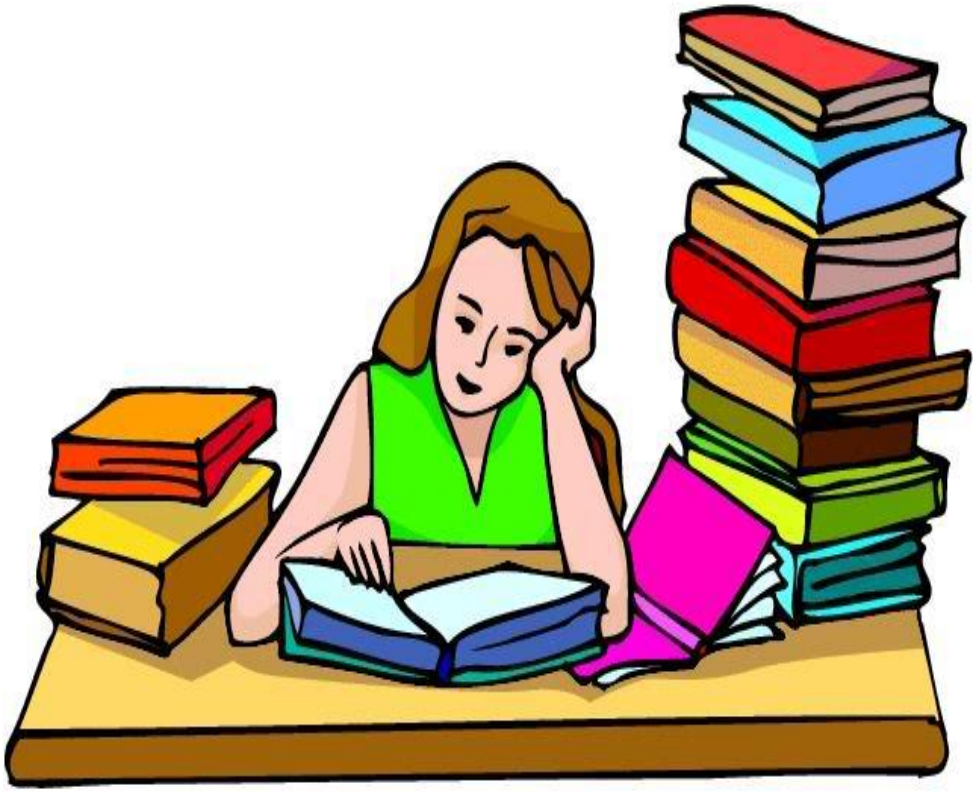
I express my deep sense gratitude to my friends ***Miss.Rajalakshmi, Mr.Asharudeen, Mr.Stromhawk Ajith, Mr.Samsudeen,*** *for their constant valuable support*

To my family, I won't be this stronger without you as my inspiration to my ***Mr.K. Chellam, Mrs.C.sumathi*** *my beloved parents! You're the reason why I keep pushing; I keep facing all the struggles, pains, and hardships. I LOVE YOU SO MUCH...*

THANK YOU ALL

CONTENTS

INTRODUCTION



LITERATURE REVIEW

RESEARCH ENVISAGED

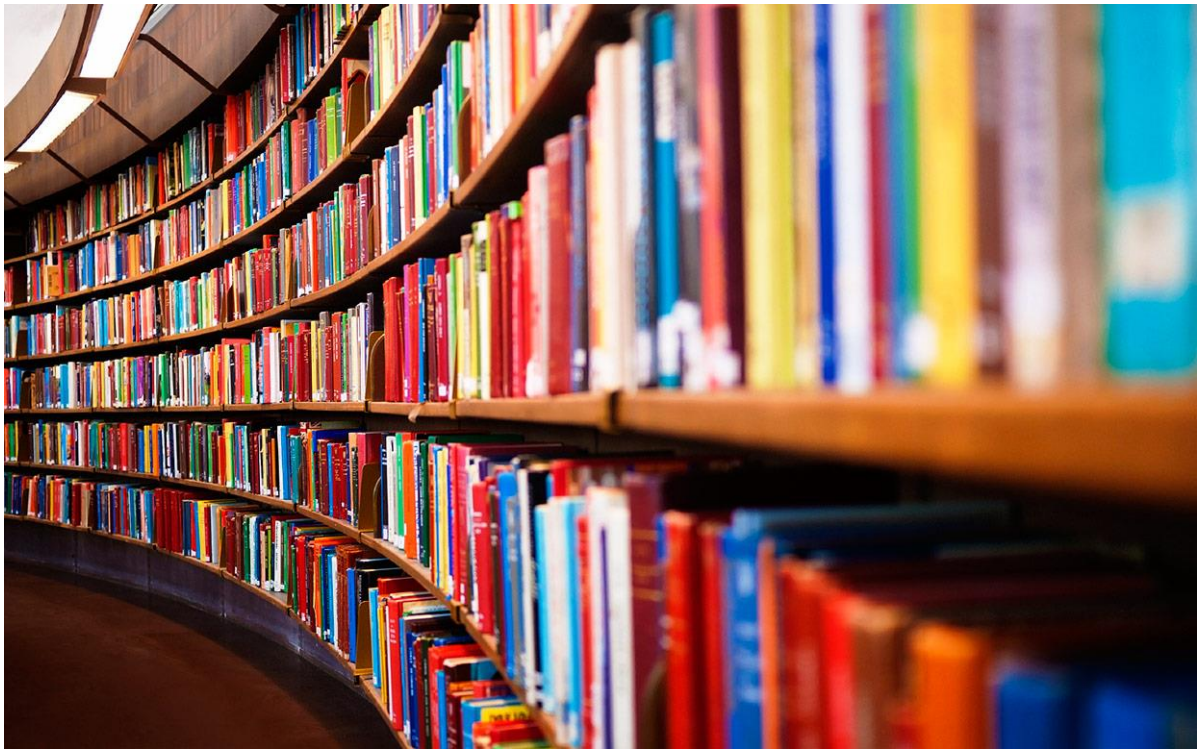
MATERIALS AND INSTRUMENTS

PLANT PROFILE

EXPERIMENTAL INVESTIGATION

RESULTS AND DISCUSSION

CONCLUSION



BIBLIOGRAPHY

Dedicated to our beloved Parents, Husband & HOD, Guide, and God

ERRATA

1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

1. INTRODUCTION OF NOVEL DRUG DELIVERY SYSTEM

The aim of NDDS is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug- delivery system should deliver drug at a rate control by the necessity of the body over a specified term of treatment.[1] These two aspects most important to drug delivery are as Follows,

I. Spatial Drug Delivery: Targeting a drug to a particular organ or tissue.

II. Temporal Drug Delivery: The drug delivery rate to the target tissue is controlled.

The prime areas of research and development for NDDS are:

- Liposomes
- Niosomes
- Nanoparticles
- Transdermal drug delivery
- Implants
- Oral systems
- Micro encapsulation / Microcapsules

1.1. Novel drug delivery system can be divided into classes. [2]

1. Sustained release drug delivery system.

2. Controlled release drug delivery system.

1.1.1. Sustained release drug delivery system

It is a pharmaceutical dosage form formulated to retard the release of a therapeutic effect such that its look in the systemic circulation is delayed and/ or prolonged and the plasma profile is sustained in duration. The onset of its pharmaceutical action is often slow, and the duration of its therapeutic effect is sustained. (e g : coated granules)

1.1.2. Controlled release drug delivery system

This system has a meaning that goes beyond the scope of sustained drug action. It manifests a predictability and reproducibility in the drug release kinetics. The release of drug Substances from a controlled release drug delivery system gains at a rate profile that is not only predictable kinetically but also reproduced from one unit to another.[3]

They are classified as follows

1. Rate pre-programmed drug delivery system
2. Activation modulated drug delivery system
3. Feedback regulated drug delivery system
4. Site targeting drug delivery system

❖ **Site targeting drug delivery system**

- Delivery of drugs to the targeted site (tissue) is complex, and it is consists of multiple steps of diffusion and partitioning. It is an uncontrolled release of drugs from the drug delivery system, but the path of drug release should be in control.
- To get read of uncontrolled drug release, drug delivery system should be site targeting specific. It is divided into three parts.

First order targeting: -

- Drugs carrier releases the drugs at the targeted site such as organ, tissue, cavity, etc.

Second order targeting: -

- Carrier releases the drugs in the specific cell such as tumours cells not to the normal cells. This is also called as the selective drug delivery system.

Third order targeting: -

- Carrier releases the drugs to the intracellular site of targeted cells.

Site targeting drug delivery system also classified as

(1) Passive targeting:

In this, drugs carrier releases the drug at the particular site due to the cause of physicochemical or pharmacological signal.

(2) Active targeting:

- It is also called as ligand-mediated targeting. In this ligand (drugs) are present on nanoparticle surface and interact with the cells or diseased cell.
- Ligand molecules are selected with the interaction of infected cell, and it should not disturb the healthy cells.
- It is designed the specific ligand for specific diseased cells. Some physicochemical properties may affect the interaction of ligands cell binding, as the ligand density, the size of nanoparticles and choice of targeting ligand for cells.
- Example of active targeting is the use of the monoclonal antibody for the treatment of cancer [4]

1.2.1. Advantage of Novel Drug Delivery system:

1. Reduce the number and frequency of doses required to maintain the desired therapeutic response.
2. Reduction in the total amount of drug administered over the period of drug treatment.
3. Reduced blood level oscillation characteristic of multiple dosing of conventional dosage forms.
4. Reduction in the incidence and severity of both local and systemic side effects related to high speak plasma drug concentration.
5. Protection from first pass metabolism & GIT degradation.
6. Maximizing availability with minimum dose.
7. Safety margin of high potency drugs can be increased.
8. Targeting the drug molecule towards the tissue or organ reduces the toxicity to the normal tissues.
9. Improved patient compliance.[5]

1.2.2. Disadvantage of Novel Drug Delivery system:

1. Administration of sustained release medication does not have prompt termination of therapy.
2. The various physiological factors such as gastro – intestinal pH, enzyme activities, gastric and intestinal transit rate, food and severity of patient's disease which interfere with the abortion of drug form the system.
3. The drug with low biological half lift can't be formulated SR formulation.
4. The potent drugs are unlike to formulate in such systems.
5. The physician has less flexibility in adjusting dosage regimen.
6. Sustained release forms are designed for normal population

1.3. NIOSOMES

- Paul Ehrlich, in 1909, initiated the development for targeted drug delivery, a drug delivery mechanism that would target directly to diseased cell
- The first niosome formulations were developed and patented by L'Oreal in 1975 Niosomes were first utilized in drug delivery for anticancer drugs.
- The main goal of a site specific DDS is not only increase the selectivity and drug therapeutic index, but also to reduce toxicity of the drug [6]

Definition

A niosome is a non-ionic surfactant –based liposome. Niosomes are formed mostly by cholesterol incorporation as an excipient. Other excipients can also be used. Niosomes have more penetrating capability than emulsions. They are structurally similar to liposomes in having a bilayer, however, the materials used to prepare niosomes make them more stable and thus niosomes offer many more advantages over liposomes. The sizes of niosomes are microscopic and lie in nanometric scale, the size ranges 10nm-100nm [7].

Advantages of Niosomes

1. The application of vesicular systems in cosmetics and for therapeutic purpose may offer several advantages.
2. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

3. Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery
4. Rate of drug administer normal vesicle in external non-aqueous phase.
5. They are osmotically active and stable, as well as they increase the stability of entrapped drug.
6. Handling and storage of surfactants requires no special conditions.
7. They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
8. They can be made to reach the site of action by oral, parenteral as well as topical routes

Disadvantages of Niosomes

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion

1.3.1. Compositions of Niosomes: [8-10]

The two major components used for the preparation of niosomes are,

1. Cholesterol

2. Non-ionic surfactants

1. Cholesterol

Cholesterol is used to provide rigidity and proper shape, conformation to the niosomes preparations.

2. Non-ionic surfactants

The role surfactants play a major role in the formation of niosomes The following non-ionic surfactants are generally used for the preparation of niosomes.

E.g.

- Spans (span 60, 40, 20, 85, 80)
- Tweens (tween 20, 40, 60, 80) and
- Brjs (brj 30, 35, 52, 58, 72, 76).

The non-ionic surfactants possess a hydrophilic head and a hydrophobic tail.

1.3.2. Structure of Niosome

A typical noisome vesicle would consist of a vesicle forming amphiphile i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of anionic surfactant such as dicetyl phosphate, which also helps in stabilizing the vesicle [11]

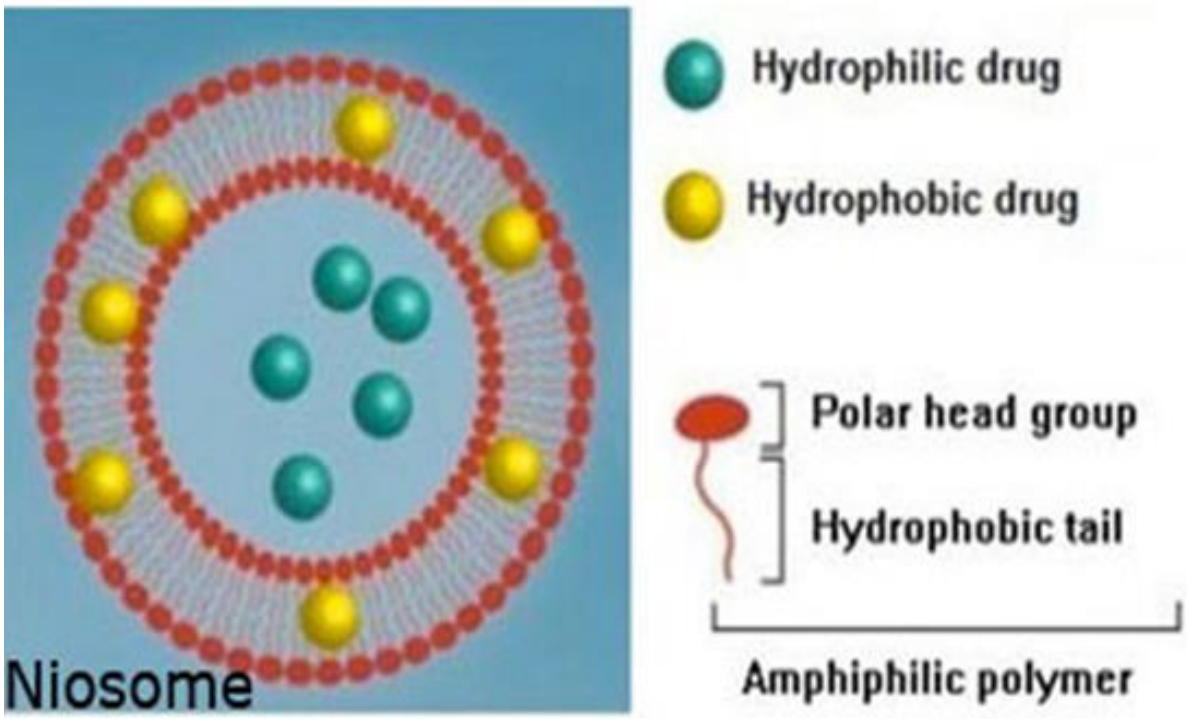


Figure 1. Structure of Niosome

1.3.3. Types of Niosomes

The niosomes have been classified as a function of the number of bilayer or as a function of size .The various types of niosomes are follows

Fig.2. Types of Niosomes

A) According to the nature of lamellarity

- Multilamellar vesicles (MLV)
- Large unilamellar vesicles (LUV)
- Small unilamellar vesicles (SUV) [12]

B) According to the size

- Small niosomes
- Medium niosomes
- Large niosomes

1.4. METHODS OF PREPARATION OF NIOSOMES [13-18]

Niosomes can be formulated by lipid hydration method, reverse phase evaporation techniques or by Trans –membrane pH gradient uptake process

Different types of niosomes can be prepared by following methods

- **Preparation of multilamellar vesicles**
  - The Bubble" method
  - Hand shaking method
- **Preparation of small unilamellar vesicles**
  - Sonication
  - French Press Method

- **Preparation of large unilamellar vesicles**
  - Reverse phase Evaporation Method
  - Ethanol Injection Method
  - Ether Injection Method
  - Homogenization
  - Trans membranes pH gradient (inside acidic) Drug Uptake Process: or Remote Loading Technique
  - Dehydration / Rehydration of small unilamellar vesicles
  - Detergent Removal Method
  - Multiple Membrane Extrusion Method
- **Preparation of multilamellar vesicles**
  - **The Bubble" method**

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (PH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas

- **Preparation of small unilamellar vesicles**
  - **Sonication**

A typical method of production of the vesicles is by sonication of solution as described by Cable. In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes.

- **Preparation of large unilamellar vesicles**
  - **Ether Injection Method**
    - ✓ This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C.
    - ✓ The surfactant mixture in ether is injected through 14-gauge needle into an aqueous solution of material.
    - ✓ Vaporization of ether leads to formation of single layered vesicles.
    - ✓ Depending upon the conditions used the diameter of the vesicle range from 50 to 1000 nm.

Multiple membrane extrusion method

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes, solution and the resultant suspension extruded through which are placed in series for up to 8 passages. It is a good method for controlling niosome size.[19]

Micro fluidization Method

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This is based on submerged jet principle in which two fluidized streams interact at ultra-high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed.

1.5. SEPARATION OF UNENTRAPPED DRUG

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: [20]

1. Dialysis

The aqueous niosomal dispersion is dialyzed in a dialysis tubing against phosphate buffer or normal saline or glucose solution.[21]

2. Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3. Centrifugation

The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.[22]

1.6. Characterization of Niosomes [23-27]

a. Scanning electron microscopy

Particle size of niosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of niosomes were studied by Scanning Electron Microscopy (SEM). Niosomes were sprinkled on to the double- sided tape that was affixed on aluminium stubs. The aluminium stub was placed in the vacuum chamber of a scanning electron microscope. The samples were observed for morphological characterization using a gaseous secondary electron detector.

b. Optical Microscopy

The niosomes were mounted on glass slides and viewed under a microscope with a magnification of 1200X for morphological observation after suitable dilution. The photomicrograph of the preparation also obtained from the microscope by using a digital SLR camera. Niosomes were sprinkled on to the double- sided tape that was affixed on aluminium stubs.

c. Measurement of vesicle size

The vesicle dispersions were diluted about 100 times in the same medium used for their preparation. Vesicle size was measured on a particle size analyzer. The apparatus consists of a He-Ne laser beam focused with a minimum power of 5 mW using a Fourier lens to a point at the center of multielement detector and a small volume sample holding cell (Su cell).

d. Entrapment efficiency

Entrapment efficiency of the niosomal dispersion in can be done by separating the unentrapped drug by dialysis centrifugation or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug

f. osmotic shock

The change in the vesicle size can be determined by osmotic studies. Niosomes formulations are incubated with hypotonic, isotonic, hypertonic solutions for 3 hours. Then the changes in the size of vesicles in the formulations are viewed under optical microscopy.

g. Stability studies

To determine the stability of niosomes, the optimized batch was stored in airtight sealed vials at different temperatures. Surface characteristics and percentage drug retained in niosomes and niosomes derived from proniosomes were selected as parameters for evaluation of the stability, since instability of the formulation would reflect in drug leakage and a decrease. In the percentage drug retained. The niosomes were sample at regular intervals of time (0,1,2, and 3months), observed for color change, surface characteristics and tested for the percentage drug retained after being hydrated to form niosomes and analyzed by suitable analytical methods (UV spectroscopy, HPLC methods etc).

h. Zeta potential analysis

Zeta potential analysis is done for determining the colloidal properties of the prepared formulations. The suitably diluted niosomes derived from proniosomes dispersion was determined using zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method. The temperature was set at 25°C. Charge on vesicles and their mean zeta potential values with standard deviation of measurements were obtained directly from the measurement.

1.7. Factors Affecting Formation of Niosomes

- Nature of surfactants
- Structure of surfactants
- Membrane composition
- Nature of encapsulated drug
- Bilayer formation
- Number of lamellae
- Membrane rigidity
- Entrapment efficiency (EE)
- Cholesterol contents

Nature of surfactants

A surfactant used for preparation of niosomes must have a hydrophilic head and hydrophobic tail. The hydrophobic tail may consist of one or two alkyl or perfluoro alkyl groups or in some cases a single steroidal group.[28]

The ether type surfactants with single chain alkyl as hydrophobic tail is more toxic than corresponding dialkyl ether chain .The ester type surfactants are chemically less stable than ether type surfactants and the former is less toxic than the latter due to ester-linked surfactant degraded by esterase to triglycerides and fatty acid *in vivo* .The surfactants with alkyl chain length from C12-C18 are suitable for preparation of niosome.[29,30]

Structure of surfactants

The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of surfactants can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation, CPP (Critical Packing Parameters) =v/lc* a0 where v = hydrophobic group volume, lc = the critical hydrophobic group length, a0 = the area of hydrophilic head group. From the critical packing parameter value type of miceller structure formed can be ascertained as given below, If CPP < ½ then formation of spherical micelles, If ½ < CPP < 1 formation of bilayer micelles, If CPP > 1 formation inverted micelles.[31]

Membrane composition

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives. In case of polyhedral niosomes formed from C16G2, the shape of these polyhedral niosome remains unaffected by adding low amount of solulan C24 (cholesterol poly-24-oxyethylene ether), which prevents aggregation due to development of steric hindrance.[31,32]

Nature of encapsulated drug

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size.[33]

Temperature of hydration

Hydration temperature influences the shape and size of the niosome. For ideal condition it should be above the gel to liquid phase transition temperature of system. Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformation.[34]

Bilayer formation

Assembly of non-ionic surfactants to form bilayer vesicle is characterized by X-cross formation under light polarization microscopy.[35]

Number of lamellae

It is determined by using NMR spectroscopy, small angle X-ray scattering and electron microscopy.[36]

Membrane rigidity

Membrane rigidity can be measured by means of mobility of fluorescence probe as function of temperature.[37,38]

Entrapment efficiency (EE)

The entrapment efficiency (EE) is expressed as

EE = amount entrapped / total amount Added × 100

It is determined after separation of unentrapped drug, on complete vesicle disruption by using about 1ml of 2.5% sodium lauryl sulphate, briefly homogenized and centrifuged and supernatant assayed for drug after suitable dilution.[39]

Entrapment efficiency is affected by following factors.

Surfactants

The chain length and hydrophilic head of non-ionic surfactants affect entrapment efficiency, such as stearyl chain C18 non-ionic surfactant vesicles show higher entrapment efficiency than lauryl chain C12 non-ionic surfactant vesicles. The tween series surfactants bearing a long alkyl chain and a large hydrophilic moiety in the combination with cholesterol at1:1 ratio has highest entrapment efficiency for water soluble drugs.[40]

HLB value of surfactants affects entrapment efficiency, such as HLB value of 14 to 17 is not suitable for niosomes but HLB value of 8.6 has highest

entrapment efficiency and entrapment efficiency decreases with decrease in HLB value from 8.6 to 1.7 30.[41]

Cholesterol contents

The incorporation of cholesterol into bilayer composition of niosome induces membrane stabilizing activity and decreases the leakiness of membrane.

Hence, incorporation of cholesterol into bilayer increases entrapment efficiency. The permeability of vesicle bilayer to 5, 6-carboxy fluorescein (CF) is reduced by 10 times due to incorporation of cholesterol.[42]

1.8. EVALUATION OF NIOSOMES

a) Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analysing the resultant solution by appropriate assay method for the drug. Where, [43]

Entrapment efficiency (EE) = (Amount entrapped total amount) x 100

b) Vesicle diameter

Niosomes, similar to liposomes, assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy and freeze fracture electron microscopy. Freeze thawing (keeping vesicles suspension at –20°C for 24 hrs. and then heating to ambient temperature) of niosomes increases the vesicle diameter, which might be attributed to fusion of vesicles during the cycle

c) In-vitro release

A method of *in-vitro* release rate study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml beaker with constant

shaking at 25°C or 37°C. At various time intervals, the buffer is analysed for the drug content by an appropriate assay method Methods for the evaluation of niosomes:

Evaluation parameter Method [44]

Morphology	:	SEM, TEM, freeze fracture technique
Size distribution	:	polydispersity index
Particle size analyzer	:	Dynamic light scattering
Viscosity	:	Ostwald viscometer
Membrane thickness	:	X-ray scattering analysis Thermal analysis
DSC Turbidity	:	UV-Visible diode array spectrophotometer
Entrapment efficacy	:	Centrifugation, dialysis, gel chromatography
In-vitro release study	:	Dialysis membrane Permeation

***In-vitro* methods for niosomes**

In vitro drug release can be done by [45]

1. **Dialysis tubing**
2. **Reverse dialysis**
3. **Franz diffusion cell**

Dialysis tubing

In vitro drug release could be achieved by using dialysis tubing. The niosomes is placed in prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, centrifuged and analyzed for drug content using suitable method (U.V. spectroscopy, HPLC etc). The maintenance of sink condition is essential.

Reverse dialysis

In this technique a number of small dialysis as containing 1ml of dissolution medium are placed in proniosomes. The proniosomes are then displaced into the dissolution medium. The direct dilution of the proniosomes is possible with this method; however the rapid release cannot be quantified using this method.[46]

Franz diffusion cell

The *in vitro* diffusion studies can be performed by using Franz diffusion cell. Proniosomes is placed in the donor chamber of a Franz diffusion cell fitted with a cellophane membrane. The proniosomes is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, and analyzed for drug content using suitable method (U.V spectroscopy, HPLC, etc) .the maintenance of sink condition is essential.

1.9. Applications of niosomes [47]

1. The application of niosomes technology is widely varied and can be used to treat a number of diseases.
2. The following are a few uses of niosomes which are either proven or under research. It is used as Drug Targeting.
3. It is used as Anti-neoplastic Treatment i.e. Cancer Disease.
4. It is used as Leishmaniasis i.e. Dermal and Mucocutaneous infections e.g. Sodium stribogluconate.
5. It is used act as Delivery of Peptide Drugs.
6. It is used in Studying Immune Response.
7. Niosomes as Carriers for Haemoglobin.Transdermal Drug Delivery Systems Utilizing Niosomes
8. It is used in ophthalmic drug delivery Other Applications: Niosomes can also be utilized for sustained drug release and localized drug action to greatly increase the safety and efficacy of many drugs.
9. Toxic drugs which need higher doses can possibly be delivered safely using niosomal encapsulation.

1.10. MECHANISM OF DRUG PERMEABILITY FROM NIOSOME

The are several mechanisms explain the ability of niosomes to modulated drug transfer across skin including

I. Adsorption and fusion on the surface of skin facilitate drug permeation
II. The vesicles act as penetration enhancer to reduce the barrier properties of stratum corneum
III. The lipid bilayers of niosomes act as a rate limiting membrane barrier for drugs

The possible mechanism for niosomal enhancement of the permeability of drugs is structure modification of the stratum corneum. It has been reported that the intercellular lipid barrier in the stratum corneum would be dramatically looser and more permeable following treatment with niosomes .The both lipids and non-ionic surfactants in the niosomes can act as penetration enhancers, which are useful for increasing the permeation of many drug. The fusion of niosome vesicles to the surface of the skin results in higher flux of the drug due to direct transfer of drug from vesicles to the skin

1.11. DISEASE OVER VIEW

SKIN DISEASES

Skin disease is a very common symptom of systemic lupus erythematosus (SLE), or lupus. Rashes are often the first visible indication of the disease.[48]

Skin diseases undermine their aspiration eroding confidence and demaging relationship social life self-image and self-esteem. Sleep disturbance and severe itching may increase Stress and further lowers the quality of life eczema and psoriasis can cause embrassement anxiety anger or depression Acne can devastate life of teenager at a time when the formation of relationship so important

The art of diagnosis in dermatology in the past was particularly emphasized by the visual experience of the skin and the skin lesions. It is true that some skin lesion can be diagnosed on the sight with the high degree of

confidence but even in such cases systematic approach is indispensable for a good dermatologist not to miss other important skin lesions, when dermatology first evolved from general internal medicine in the nineteenth century

There is usually some good reason for tropical treatment ,the drug is irritant or sensitizing or does not adequately penetrate the skin The base In which drug is applied to the skin is important

Bacterial skin infections

Bacterial Skin Infections or Pyoderma Bacterial skin infections or pyoderma are common in most developing countries.[49]

Generally these infections arise as primary infections of the skin known as impetigo or as secondary infections of other lesions such as scabies or insect bites. The usual bacterial causes are Group A *streptococci* or *Staphylococcus aureus.*[50]

Bacterial infections are common in communities. In many cases, no bacteriological confirmation is available from cultures, but surveys show that Group a streptococci account for a substantial number of cases which is not often the case in similar infections in temperate climates, where *S. aureus* dominates. This finding carries implications for the selection of treatment options. The reasons for this finding are not clear, although humidity and heat are associated with increased risk of bacterial skin infection. In addition to these superficial infections, S. aureus also causes folliculitis or hair follicle infections and abscesses. Rarer causes of skin infection in developing countries include cutaneous diphtheria and anthrax, as well as necrotizing infection caused by Vibrio vulnificus. Bacterial infection causes irritation and some discomfort.[51]

In some cases, the infection penetrates deep down through the epidermis, causing a necrotic ulcer a condition known as ecthyma. However, some evidence suggests that streptococcal infection may cause additional long-term damage through the development of prolonged proteinuria, as described earlier in relation to scabies.[52]

Fungal Skin Infections

Fungal infections that affect the skin and adjacent structures are common in all environments. They include infections such as ringworm or dermatophytosis; superficial candidiasis and infections caused by lipophilic yeasts and Malassezia species;

Fungal infection are termed mycoses and in general can be divided in to superficial infection and systemic infections many of the fungi that can cause mycoses live in association with man as commensals are present in his environment. The serious superficial infections were relatively uncommon and systematic infections very common. [53]

Fungi are the most common parasites led by the ubiquitous yeast, *candida albicans*. Candida organisms are natural residents of the body, but they are normally found in harmless proportion. The body's natural defence against fungal infection is its resident population of normal bacterial flora which prevents invasion by foreigners. But yeast is the impervious to the antibiotics that wipe out bacteria when friendly bacteria wiped out along with unfriendly one by these drugs.

Fungal infections can also affect other parts of the body. Onchomycosis fungal disease of the toe nails and finger nails particularly affected the elderly one has increased by a factor in the last 20 year.

Common fungal diseases

- *Candidiasis*
- *Pityriasis versicolor*
- *Tinea pedis*
- *Tinea manuum*
- *Tinea unguium*
- *Tinea capitis*

1.12. INTRODUCTION OF *SENNA ALATA LINN*

- *Senna alata Linn*. (English: Ringworm shrub, Sanskrit: Dadrughna, Hindi: Dadmurdan) is an erect shrub growing up to 3-4.5 m in height.[54]
- It shows large paripinnately compound leaves. The flowers are golden yellow in colour and appear in racemes.[55]
- Pods are flat, dark brown with many seeds. The plant is cosmopolitan in distribution.
- It is found wild as well as the cultivated ornamental plant throughout India. Various parts of S. alata are used for diverse healing actions

PHARMACOLOGICAL ACTIVITIES

The research data indicate that *Senna alata* possesses enormous pharmacological values which support its various traditional uses for the management of health problems. The most important are: [56]

Antimalarial activity:

Senna alata L. leaves exhibited in vitro antimalarial activities against Plasmodium falciparum in ethylene Glycol-water 3:7 solvent; with IC_{50} values below 1µg/ml value of 17.270µg/ml. The antimalarial activity of this plant is doubtless due to its alkaloids, quinones and terpenes. In fact, in the chemotherapy of malaria, most molecules belong to the class of alkaloids these studies have provided scientific evidence for traditional usage of Senna alata as effective remedy for the treatment of malaria in many African countries.[57]

Anti-cancer and antitumor activity:

Study designed to investigate the sub-acute toxicity, in vivo antioxidant and antitumor activity of aqueous ethanol extract of *Senna alata L* on bearing carcinomatous cells on Nude mice the results of in vivo antitumor activity of extract of Senna alata observed. Hence, the leaf extract of Senna alata can be used to cure the breast cancer. Evaluated leaf extracts of Senna alata for their potential antitumor properties in vitro MTT assays were used to examine the cytotoxic effects of crude extracts on five human cancer cell lines, namely MCF-7, derived from a breast carcinoma, SK-BR-3, another breast carcinoma, T24 a bladder carcinoma, Col 2, a colorectal carcinoma, and A549, a non- small cell

lung adenocarcinoma. Hexane extracts showed remarkable cytotoxicity against MCF-7, T24, and Col 2 in a dose-dependent manner. This plant extract had also proved its effectiveness against Leukaemia cells.[58]

Anti-inflammatory activity:

The anti-inflammatory mechanism of a hexane extract of *Senna alata L* was investigated in Complete Freund's Adjuvant (CFA) arthritis, as a chronic model of inflammation

The CFA model was created by the injection of 0.5ml CFA into the synovial cavity of the right knee joint of hind leg of rats. Changes in knee joint swelling, cartilage integrity and synovial fluid leukocyte counts were assessed in response *Senna alata L* to treatment.[59]

Anthelmintic activity:

Alcohol extract from the leaves of *Senna alata L* and *Typha angustifolia* were investigated for their anthelmintic activity against Pheretima posthumous and Arcadia galls In conclusion, the traditional claim of leaves of *Senna alata L* as anthelmintic have been confirmed as the leaf extracts displayed activity against the worms used in the study.[60]

Antioxidant activity:

Sarkar et al carried out a study to evaluate in vivo the antioxidant and antitumor activity of the aqueous-EtOH extract of leaves of *Senna alata L.* Antioxidant potential was found to be more in extract as compared to control. The results of this study clearly indicated that the extracts of *Senna alata L* could be used as a potential source of natural antioxidant agents. The antioxidant activity of crude methanol extracts from the leaves, flowers and pods of *Senna* alata.[61]

Hepatoprotective and hepatocurative activities:

Hepatoprotective activity of the Infusion of the dried leaves of *Senna alata* (ICA) was studied against Paracetamol induced hepatic injury in albino rats Pre-treatment of the Infusion (ICA) reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate trans peptidase (GGTP). Histopathological observations also revealed that pre-treatment with ICA protected the animals from Paracetamol induced liver

damage. The results indicate that the leaves of *Senna alata* possess the hepatoprotective activity. This property may be attributed to the flavonoids present in the leaves of this plant. The effect of oral administration of aqueous extract of leaves of *Senna alata* in various doses (2.5-20.0 mg/kg) for seven days, on hepatic induced damage by administration of 45% of ethanol (20ml/kg) and CCl4 (0.1 ml/kg) in rats of the use of *Senna alata* in the traditional medicine for the treatment of cirrhosis and hepatitis.[62]

Antibacterial and antifungal activities:

Antimicrobial studies showed that the isolated compound from *Senna alata* seeds successfully inhibited Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus Candida albicans and Aspergillus Niger. Chemical investigation of the bioactive constituents from the seeds of Senna alata resulted in the isolation of a new cannabinoid alkaloid (4-butylamine 10-methyl-6hydroxy cannabinoid dronabinol). The antimicrobial observation of the above compound against these pathogens showed that the bioactive compound could be responsible for the activity of the plant and its use in traditional medicine. These findings also justify the use of *Senna alata* in the treatment of skin infections such as eczemas, ringworms, boils, carbuncles, breast abscess, infantile impetigo, sores and wound in herbal medicine and its use as an ingredient in the formulation of medicated and antiseptic soaps.[63]

Cardio protective activity:

Research work designed to evaluate the cardio protective potential of the *Senna alata* leaves against doxorubicin induced cardiac toxicity in rats *Senna alata* leaves methanolic extract has significant cardio protective activity. Preliminary phytochemical investigation of *Senna alata* leaves methanolic extracts shows presence of flavonoids, tannins, glycosides. Thus, the strong antioxidant and cardio protective effect of the extract could be attributed to the presence of bioactive constituents present in the extract. [64]

Anti-diabetic and antihyperlipidemic activities:

A study aimed to depict the therapeutic effect of *Senna alata* leaf aqueous extract on oxidative stress in aorta as well as heart of streptozotoc in hyperglycaemic rats was undertaken by Two days after diabetes induction effect.

Thus, one of the possible antidiabetic mechanisms of action of *Senna alata* is by inhibiting carbohydrate digestion.[65]

Bronchorelaxant activity:

Aqueous-ethanolic extract of *Senna alata* (AECal) and its derived fractions obtained through liquid-liquid fractionation were evaluated for their bronchorelaxant effect activity of rats tracheas in the presence of tested materials, as well as its modifications with different inhibitors and blockers, was isometrically recorded. In animals pre-treated with the extract, the percentage of Induced DNA damage decreased. The results suggest that (1) muscarinic receptors contribute at least in part to the relaxant effects of AECal; (2) AECal interferes with membrane polarization. Evaluation of isolated bioactive molecules from plant extract eliciting tracheorelaxant effect may give new investigational and treatment tools in broncho respiratory pharmacology. This study provides sound mechanistic basis for the use of *Senna alata* in asthma-induced bronchospasm.[66]

Antiviral activity:

Mohamed et al investigated the antiviral activity of five extracts (methanol, chloroform, ethyl acetate, n-butanol, and aqueous) from *Senna alata* leaves against rotavirus (RV) infection *in vitro* and *in vivo*). *In vitro*, all extracts prevented the cytopathic effect (CPE) of RV, as demonstrated in an MTT colorimetric and karber methods, with therapeutic index (IT) ranged from 22.8 to 0.02 and reduction in virus titres ranged from 4.25 and 0.25 log10TCID50. The methanol extract was the stronger than the other extracts against RV replication. In vivo antiviral activity of the methanol extract against rotavirus was evaluated, using a mouse model. Orally administered methanol extract at 100 mg and 50 mg /kg body weight/day significantly reduced virus yield in the small intestine as well as it reduced mortality, severity and duration of diarrhoea after infection for 7 days. In addition to this, extract protects the intestinal tissues from damage resulted from RV infection when compared with the untreated infected control. These results clearly shows that *in vitro* and *in vivo* infection with RV can be effectively treated by the methanol extract of *Senna alata* leaves. The antiviral activity of methanol extract of *Senna alata* may be attributed to the presence of Saponins. The anti-rotavirus activity of Saponins has been well documented *in vivo*. Thus,

the present study has shown that the methanol extract of *Senna alata* recovered the rotavirus gastroenteritis by coordinating antiviral and anti-inflammatory effects.[67]

Reported toxicity:

Investigated the acute and sub-acute toxicities of hydro-ethanolic extract of leaves of *Senna alata* in Swiss mice and Wister albino rats The mice were divided into 6 groups of 10 animals and each group received once by intra gastric gavages 0, 4, 8, 12, 16, 20 times 1000 mg/kg dose Med. Sci. of extract. Distilled water served as the control. For the sub-acute toxicity, three groups of 10 rats (5 males and 5 females) were treated per so with distilled water (control), 500 or 1000 mg/kg of extract every 48 h for 26 days. At the end of treatment blood sample and 20% liver homogenates were collected for biochemical analyses. The results indicated that the medium lethal dose (LD50) was about 18.50 g/kg of body weight. Significant variation ($P<0.05$) of the body weight was observed after 26 days of treatment, in some biochemical index of serum and 20% liver homogenates (glutathione , alkaline phosphatase (APL), aspartate aminotransferase (AST)), haematological parameters (platelet) also in the female relative weight of heart of rat. Some of parameter investigated in this study showed dose responsive. The histo pathological study of the liver did not show any features after the treatment but, the extract seems to ameliorate the liver architecture. In another study, no death and no clinically significant changes were recorded in mice which consumed this plant extract. The maximum non-lethal dose was more than 1687.5 mg/kg in animals. No significant changes were observed in body weight, tissues morphology, biochemical and haematological parameters at doses above or equal to 2779.5 mg/kg body weight.[68]

2. LITERATURE REVIEW

2.1 STUDIES ON *SENNA ALATA* LEAF

Neil Alejandra Pinarok, N.A.A *et al.,* (2017) Developed *Senna alata L.* leaf extract was used to develop an antifungal ointment with two different formulations namely; Simple ointment and Emulsifying ointment using Jatropha curcas L. seed oil as base.. *In vitro* antifungal assay established that *Senna alata L.* ointment whether simple or emulsifying has antifungal effect against common fungi. Stability tests of the processed ointments have stable characteristics in normal conditions, but slightly changes when exposed to higher temperatures other than normal temperatures, especially its pH, and weight. Jatropha curcas seed oil extract can be used to manufacture cosmetic products.[69]

Vigbedor Bright Yaw *et al.,(* 2015) Investigated the stem bark of A. Africana and the leaves of *Senna alata L.* for their antimalarial activity against the 3D7 strain of the Plasmodium falciparum parasite. by employing the WHO micro test assay (Mark III). Compared with Artesunate-amodiaquine. The standard drug, Artesunate-amodiaquine was the most active with an Inhibitory Concentration (IC50) of 0.313µg/ml this was followed by Afzelia Africana, and Senna alata with IC50 values of 2.954µg/ml, and 17.270µg/ml respectively.[70]

Raphael M. Mordi *et al*., (2016) Evaluated the antimicrobial properties of the ethanol and aqueous extracts of *senna alata* leaves.by Agar diffusion method the muella-hinton agar for the bacteria and potato dextrose agar for the fungi. phytochemical screening revealed the presence of cardiac glycoside, reduced sugar in equal concentration in both extracts while flavonoids, terpenoids, Saponins and phenolic were in slightly higher concentrations in the ethanol extract than in the aqueous extract. The antimicrobial effects produced by the extracts were dose dependent at the tested doses; 1000mg/ml, 500mg/ml and 250mg/ml.[71]

Addai mensah donkor *et al.,* (2016) studied antibacterial activities of the extracts of medicinal plants, Senna alata, Ricinus communis and Lannea barter. Bacteria strains such as Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli are known wound and skin disease causing bacteria.. Phytochemical screening of the crude extracts of Senna alata, revealed

the presence of some bioactive components, including tannins, Anthraquinones, Saponins, flavonoids and alkaloids. The extracts showed inhibition on test organisms and were in the range of 12.0 ± 2.83mm to 36.0 ± 2.12mm.The MIC values obtained for the entire test ranged from 3.13mg/ml to 12.50mg/ml and MBC values were found to be 200mg/ml minimum and 400mg/ml maximum.[72]

Senga Kitumbe *et al., (*2016) formulated a dermal ointment from the whole leaves of *Senna alata Linn* in order to improve the traditional preparation used against dermal diseases. In comparison with the aqueous extract of *S. alata* leaves medicinal properties, gentamicin and ketoconazole discs were used as controls. the herbal ointment demonstrated higher antifungal activity than antibacterial activity based on the zones of inhibition recorded for all the concentrations. The chromatographic fingerprints established and quantitative analyses conducted in this investigation are worth considering for the quality control of the herbal ointment formulated.[73]

M. S.Rahman *et al.,(*2015) isolated isoflavone from the leaves of Senna alata , 2, 5, 7, 4′-tetrahydroxy isoflavone with the help of column and thin layer chromatography by using a gradient mixture of organic solvents with increasing polarity. The compound was characterized on the basis of UV, IR, and 1H-NMR, 13C-NMR and Mass spectrometry and confirmed the compound belonged to the isoflavone series.[74]

Dr. D. Victor Arokia Doss *et al.,* (2015) prepared Senna alata juice of fresh leaves of recognized by local healers as a remedy for parasitic skin disease and is used in the treatment of many eruptive and pustular skin condition by simply rubbing the crushed leaves either alone or mixed with oil on the skin. Root is taken in Nigerian and Guinea with rock salt and other dry medicinal plants is taken in Nigeria thrice weekly on an empty stomach for effective treatment of chronic gonorrhoea. the existence of bioactive compounds with free radical scavenging activity, anticancer property and antidiabetic activities.[75]

Meenu priya *et al.,* (2014) carried out the phytochemical screening and bioassay of Senna alata leaf extract and its skin hyperpigmentation activity. Medicinal plants of Indian origin play a significant role in treating a broad spectrum of human

diseases natural products seem a reliable alternate that's considered safe and effective. The phytochemical screening and analysis of *Senna alata* leaf extract against hyperpigmentation of skin due to spray allergy and under eye darkness. The efficacy of hydro ethanolic extract of Senna alata applied to under eye dark circles and hyper pigmented under arms due to regular usage of body spray. The results are highly promising on regular application twice a day for 20 days. Visible difference is observed within one week of application and normal skin color is obtained within a month's time.[76]

Rama Swamy Nanna *et al.,* (2015) evaluated anti-diabetic activity and antihyperlipidemic evaluation of leaf extracts of senna alata in alloxan induced diabetic rats Senna alata is an ethno medicinal plant belonging to the family Fabaceae. A number of bioactive constituents which contribute to the medicinal properties are attributed to the species. The anti-diabetic activity attributed to the species and also to evaluate the anti hyperlipidemic potential of the Species using various standard experimental models available. Acute toxicity studies of aqueous leaf extracts of S. alata were performed up to a dose of 2500mg/kg bodyweight of rats and a dose of 200 mg/kg was selected. Aqueous leaf extracts of S. alata showed a significant ($P<0.01$) anti diabetic and anti hyperlipidemic potential in alloxan induced diabetic rats within 15 days of induction of diabetes and the antidiabetic potential of the species may be due to the presence of flavonoids.[77]

Pratiwi Wikaningtyasthe *et al,.(*2015) evaluated antibacterial activity of *senna alata* leaf extract and fraction towards MRSA (methicillin resistant *staphylococcus aureus)* and its mode of action. The increasing prevalence of healthcare-associated MRSA infections need new approach to overcome of growing problems. Medicinal plants are promising as the most valuable resources for antibiotic development. Senna alata had shown antibacterial effect in Str. pyogenes and S. aureus the antibacterial activity calculated based on Minimum Inhibitory Concentration (MIC) using Mueller Hinton broth in micro dilution method and the mode of action was conducted using Scanning Electron Microscopy (SEM). Phytochemical screening of dried Senna alata leaf and its extract showed the presence of flavonoid, alkaloid, Saponins, Quinone, tannin, and sterol. The antibacterial activity showed the MIC value of the extract against MRSA was

512µg/mL. The ethyl acetate fraction showed the best MIC value at 256µg/mL. The SEM observation of MRSA treated by ethyl acetate fraction of Senna alata showed membrane shrinkage. Senna alata was promising to be developed as antibacterial agent especially MRSA strain.[78]

Mulham alfatama *et al., (*2012) formulated Microencapsulation of *Senna alata* and its antifungal and antimicrobial activities. This extracts *S. alata*, followed by microencapsulation by solvent-evaporation using biodegradable PLGA. The microsphere is envisaged to release the extract in a controlled manner allowing more convenient mode of administration when treating related skin infection. Several variables had been investigated including different types of surfactants and buffer systems and different homogenization times for primary emulsion. It was found that, most of the surfactants employed resulted in low encapsulation efficiency (<10%). However with 3% poly vinyl alcohol (PVA), slightly higher encapsulation efficiency of 11% was achieved, with size distribution of 7-13 µm. The encapsulation efficiencies were significantly improved (45-64.4%) when the hardening tank was buffered to pH 7, with minimal effect on particle size. Additionally, in-vitro controlled release formulation of S. alata microsphere was demonstrated for duration of almost a month.[79]

Selvi V*et al.,* (2012), studied on antimicrobial activities from flower extract of Senna alata Linn. The plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientist's worldwide. The medicinal usefulness of the plant *Senna alata Linn*. *S. alata* is an ornamental shrub or tree growing up to 12 m high and widely available in the tropics, These trees are used to treat diarrhoea, dysentery and other gastrointestinal problems. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases. The decoction of the stem bark and roots are used to treat urinary tract infections, bronchitis and asthma. the microbial activity of methanol, chloroform and petroleum ether extracts of flowers of *Senna alata* Evaluated for potential antimicrobial activity against bacterial and fungal strains. The fungal isolates tested include: Epidermatophyton floccose, Microsporium gypseum and Trichophyton mentagrophyte and the Bacterial isolates tested include: Escherichia coli. The antimicrobial activity was determined the extracts using agar well diffusion

method. The flowers were shade dried and then homogenized to fine powder by a mechanical grinder. They were extracted using different solvents such as by methanol, chloroform and petroleum ether by soxhlet apparatus. The zone of inhibition was measured.[80]

Silver Ighosotu *et al.,* (2013) investigated the effects of Senna alata L. aqueous leaf extract on the germination of Corchorus olitorius the effect of different concentrations of Senna alata crude aqueous leaf extract on the germination of Corchorus olitorius S. alata aqueous leaf extract (10, 30, 50, 75 and 100% C) used in this study caused significant ($P < 0.05$) decrease in the total percentage and germination rate of C. olitorius. Pre-soaking of C. olitorius seeds in C. alata crude aqueous leaf extract led to an increase in the lag-phase period preceding germination of the seeds in a concentration-dependent manner. The total percentage and rate of germination decreased as extract concentration increased. All concentrations caused a consistent decrease in absolute rate and percentages of germination compared to the control.[81]

Mohammed I Ali *et al.,* (2017) studied Leaf extracts of *Senna alata* L traditionally used for treatment of a variety of diseases. Chloroform fraction of leaves was evaluated for its potential antitumor properties in vitro. MTT assay was used to examine the cytotoxic effect on three human cancer cell lines namely HepG2, MDA-MB-231 and Caco2. Chloroform fraction showed remarkable cytotoxicity against HepG2 cells with IC50 = 37.4 µg/ml at exposure time 48 h. The fraction exhibited weak anti-proliferative effect on Caco2 and MDA-MB-231 cells (8.2% and 11.6% respectively), with IC50 values >100 µg/ml. DPPH free radical scavenging activity of the fraction (100 µg/ml) revealed weak antioxidant activity (7.8%). Further bioassay-guided fractionation of the cytotoxic fraction led to the isolation and characterization of three Anthraquinones (rhein, aloe-emodin and emodin).[82]

R. P. Senthil kumar *et al.,(*2013) carried out phytochemical screening and antibacterial evaluation of the leaf, flower and seed coat extracts of Senna alata L Preliminary studies on the phytochemistry and extract of diethyl ether, chloroform and acetone of Senna alata L leaf, flower and seed coat were examined for antimicrobial properties. Extract tested against clinical isolated of Pseudomonas sps, Escherichia coli, Staphylococcus sps, Shigella boydii and Salmonella sps. The

zone of inhibitions produced by the extracts in disc diffusion assay against the test microorganisms, the produced zones that measured in mm. Preliminary phytochemical analysis showed that the extracts contained tannin, flavonoid, terpenoids, cardiac glycosides, steroids and terpenoids, absence of alkaloids.[83]

Panichayupakaranant *et al.,* (2004) isolated the antioxidant constituent from *Senna alata* L. leaves using DPPH radical scavenging assay The DPPH radical scavenging assay-guided isolation, the methanol extract of C. alata leaves was separated by silica gel vacuum chromatography and Sephadex LH-20 gel filtration chromatography afford a light yellowish powder (CA1), which was identified as kaempferol. This compound exhibited antioxidant activity (ED50 9.99 µM) that was six times stronger than that of BHT (ED50 57.41 µM) and fifty eight times stronger than that of emodin (ED_{50} 578.87 µM).[84]

Da FL, Keugni *et al,* (2018) Evaluated anti-inflammatory and protective effect of *Senna alata* Linn Leaves.The anti-inflammatory and protective effect of dichloromethane extract of Senna alata Linn. leaves (CF-AECal) has been studied according to Freund's adjuvant-induced arthritic rat models. CF-AECal 50 mg/Kg, 100 mg/Kg and dexamethasone 1 mg/Kg were the doses of drugs receipt by rats. CF-AECal 100 mg/Kg showed a substantial anti-inflammatory and protective effect. CF-AECal 50 and 100 mg/Kg produced antioxidant effects in Freund's adjuvant-induced paw oedema. Indeed, antioxidant enzymes are maintained more or less that their normal rate. These antioxidant effects protect the liver, the kidney and the spleen highly ($p< 0.001$). CF-AECal 50 and 100 mg/Kg lead to a significant decline in the rate of serum enzymes (AST, PAL and CREA) and a decrease of the serum concentration of total bilirubin and protein compared to animals of the control group ignited. At the doses of the extract used, the leukocyte infiltration is reduced in the cutaneous cloths of the paws of rats.[85]

Ikechukwu *et al.,* (2014) Investigated The efficacy of crude extracts of Senna alata in the improvement of vegetative and reproductive growth in Celosia argentea. Fresh leaves of S. alata were blended with a homogenizer in 1 litre of distilled water. The resultant green paste was filtered under suction. Different concentrations (75%, 50%, 40%, 30%, 25%, 12%, 10%, and 5%) were prepared from the 100% crude extract. Seeds of C. argentea were pre-soaked in these

different concentrations including a control (0%) and planted out after 24 hours. Results showed that seedling height, leaf area, dry weight and leaf area ratio were promoted and enhanced by pre-soaking seeds in the extract. Seedlings raised from seeds pre-soaked in water (control) however, flowered earlier (8 weeks) than the treatments (10 weeks in 75% and 100%). Pre-soaking seeds of C. argentea in crude extracts of S. alata before planting is recommended for optimum production of the leafy vegetable. The procedure is cheap and easily implementable by resource-poor farmers who are the main growers of C. argentea.[86]

Chris. A. Alalor *et al,.* (2012) Evaluated antibacterial activity of Herbal ointments formulated with Methanolic extract of *Senna alata* LThe preliminary in vitro antibacterial activity of the methanolic extracts of sun-dried leaves of Senna alata against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa using the Agar cup plate method. Herbal ointments were prepared by incorporating the methanolic extract of Senna alata (10 % w/w) into aqueous cream, emulsifying ointment and simple ointment bases and evaluated for their in vitro antibacterial efficacy. The formulation containing Senna alata extract in aqueous cream showed comparatively better antibacterial activity than the other formulations in the following order: aqueous cream > emulsifying ointment > simple ointment > crude extract. The herbal ointment also compared favourably with a commercial brand of Gentamicin ointment. has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions.[87]

Okooboh *et al.,* (2013) studied phytochemistry and antimicrobial activity of the leaf of senna alata linn. The leaves of were extracted with n-hexane and ethyl acetate using the soxhlet method. Preliminary phytochemical screening of the extracts revealed the presence of free Anthraquinones, flavonoids, steroids and Saponins. The n-hexane crude extract exhibited some antibacterial activity against Yersinia enterocolitica, streptococcus pneumonia and salmonella typhi. Antifungal activities against Microsporium audouini and Trichophyton meritagrophyta were also exhibited. A synergic test of n-hexane and ethyl acetate extracts showed an improved sensitivity against Shigella sormei and strep. Pneumonia. A confirmatory

phytochemical analysis performed on the most mobile TLC isolate (RF 0.94) from the n-hexane extract revealed the presence of steroidal Saponins. This was found to be active against strep. Pneumonia.[88]

Adlis Santoni *et al,.* (2016) Performed structure elucidation of anthraquinone derivate from *senna alata Linn* and Antioxidant Activity This plant has traditionally been used as an anthelmintic, thrush. Laxative, anti-parasitic, herpes, syphilis, scabies and other skin diseases. The isolated anthraquinone compound from the leaves of the plant. Extraction was done by soxhletation method, while purification was done by vacuum liquid chromatography with silica gel stationary phase and gradually eluted using Step Gradient Polarity (SGP) method by using the solvent n-hexane, ethyl acetate and methanol. Structure elucidation done by ultraviolet, infrared and 1H-NMR spectroscopy. Compound was isolated from ethyl acetate fraction as an orange powder The isolated compound is anthraquinone derivate. Testing the antioxidant activity of the ethyl acetate fraction shown IC50 = 310 µg/ mL and classified as active antioxidants.[89]

Douye V *et al.,* 2014 studied antimicrobial activity of ethanol extract of *senna alata* leaves against some selected microorganism. *Senna alata* is an underutilized shrub growing in many parts of the world and is known for its traditional use in the treatment of dermotophytes and other related ailment. The ethanol extract of *Senna alata* leaves was evaluated against some dermotophytes (Malassezia pachydermatis Malassezia furfure and Malassezia restrict, Malassezia globosa) and gastrointestinal bacterial pathogens (Salmonella Typhi, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, and Klebsiella spp). The extract was tested using well diffusion technique against pathogens and found effective against the selected pathogens. The highest zone of inhibition was observed against Klebsiella spp (27.4mm), followed by S. Typhi (26mm) and P. auriginosa (26mm), P. mirabilis (21.7mm) and E. coli (19.5mm). In case of fungi, it was most effective against M. globosa (19.7mm) and M. pachydermatis (17mm). It was not effective against M. furfure and M. restrict a. Thus*, S. alata* is a good antimicrobial agent against bacterial and fungal pathogens of humans.[90]

K. kavipriya *et al.,* (2018) detected FTIR and GCMS analysis of bioactive Phytocompounds in methanolic leaf extract of *Senna alata* by using soxhlet

apparatus. FTIR and GCMS analysis were done to this plant extract to find out the bioactive Phytocompounds. The FTIR results of this plant extract showed peaks indicate the presence of the bioactive compounds such as sulphates, sulphonamides, sulfones, sulfonyl chlorides, sulphates, sulphonamides, alkanes, aromatic, aromatic, alkenes, ester, alkenes, ketenes, isocyanides, isothiocyanates, acetylene, nitrile, phosphine, phosphine, aldehyde, alkane, amide, alcohol and alcohol. The GCMS results showed peaks. The retention time (RT) of all these peaks indicate the presence of functional group such as 1-Butanol, 3-methyl-1,6-Anhydro-.beta.-D-glucopyranose (laevoglucose), 3-O-Methyl-dglucose, Oxirane, 10-Methyl-E-11-tridecen-1-ol propionate, l-(+)-Ascorbic acid 2,6-dihexadecanoate, (R)-(-)-14-Methyl-8-hexadecyn-1-ol, Oleic Acid , Vitamin E acetate and 1,2-Bis(trimethylsilyl)benzene.[91]

Kudatarkar NM *et al.,* (**2018)** studied Pharmacological Screening of *Senna alata* leaves on Colorectal Cancer. *Senna alata* extract (CAE) was standardized by HPTLC. Upon oral administration of 2 g/kg, *Senna alata* leaves extract (CAE) to female rats, mortality was not observed, and animals did not show any signs and symptoms of toxicity. Upon treatment for four weeks the rats did not show any cumulative toxicity profiles. Haematological parameters showed significant differences between the groups. In ACF count and colon length by weight ratio, there was significant difference found. In histopathological examination, it was found that there was formation of dysplasia in standard and post treatment and malignancy was found in disease control and co-treatment group.[92]

Solomon Oluwole Oladeji *et al.,* (**2016)** obtained Mass spectroscopic and phytochemical screening of phenolic compounds in the leaf extract of *Senna alata* using soxhlet apparatus and the concentrated extracts were purified using column chromatography; the fractions were eluted and screened for their phytochemical and the mass spectroscopic analysis was performed using a mass spectrophotometer. The antimicrobial activity was carried out using agar disc diffusion method. The phytochemical analysis revealed the presence of important secondary metabolites such as anthraquinone, flavonoid and Saponins while steroids was absent in the leaf extracts. The molecular ions of 250, 250, and 222 were obtained from the mass spectra. This showed the presence of methaqualone,

cinnamic acid and isoquinoline. Ethanolic extracts showed a higher antimicrobial activity when compared with the methanolic extracts but less activity when compared with the standard used (amoxicillin).The presence of these phytochemicals could be responsible for the observed antifungal and antibacterial activities on the susceptible organisms studied of the plant and also can be a natural source of antimicrobial substances of high importance.[93]

G.R.Nalini *et al.,* 2017 compared of antifungal sensitivity of *senna alata* leaf and flower extracts against Candida albicans. The plant Senna alata is a shrub that has various uses ranging from mild to severe infectious and non-infectious diseases. The study was comparing the antifungal efficiency of the methanolic leaf and flower extracts of Senna alata against Candida albicans. The leaves and flowers or the plant were collected, dried, extracted by continuous hot percolation method using methanol. The extract was used for antifungal sensitivity testing by well diffusion method at different concentrations. At 20 percent, the zone of inhibition for the leaf was 1.90 ± 1.14 and for flower was 1.10±1.58. At 40% level, the zone of inhibition for leaf was 4.10 ± 0.64 and for flower was 2.80±0.84. While at 60% level, zone of inhibition for leaf extract and flower was 5.80 ± 0.31 and 3.60 ± 0.54, respectively and at 80% level, the zone of inhibition for leaf extract and flower was 7.90 ± 1.10 and 4.40 ± 1.10, respectively. Whereas, the pure extract of leaf depicted 8.80 ± 0.46 and flower depicted 6.20 ± 0.68 zone of inhibition. the leaf extract has better anti-fungal properties than the flower due to the presence of more phyto-pigment anthraquinone in leaves than flower petals.[94]

Mitra Prasenjit *et al.,* (**2016)** Isolated and characterization of a compound from the leaves of senna alata linn.by solvent extraction, acid hydrolysis, chromatography followed by crystallization.Infra-red spectroscopy, mass spectroscopy and nuclear magnetic resonance studies revealed that the isolated compound was chemically 1, 3, 5-trihydroxy-7-methylanthracene-9, 10-Dione.[95]

M. Wuthi-udomlert S *et al.,* (2003) studied antifungal activities of *senna alata* extracts using different methods of extraction Senna alata (L.) Roxb. Has been used for a long time for the treatment of tinea versicolor and ringworm infections. Conventional methods using various solvents and soxhlet extraction yielded fractions with different properties. Lyophilisation of the aqueous extract might limit

the ingredients obtained. However, both methods differed in their yields: in the obtained percentage, appearance, properties and time and cost consumed. In addition, sonication was an alternative to acquire the active ingredients from the plants. the extracts were investigated for their antifungal activities. On the basis of inhibitory zone, activities against dermotophytes and Candida albicans 36 and 26 clinical isolates, respectively, were established by an agar diffusion method. The extracts A, B and C (20 mg, each), D and E (80 µg, each) inhibited the dermotophytes by 13.8, 9.9, 21.9, 8.2 and 7.5 mm and C. albicans by 18.8, 10.7, 14.1, 10.1 and 7.2 mm, respectively. From TLC, the crude ethanol and ethanol sonicated extracts (A and D) of S. alata were shown to contain rhein (anthraquinone aglycones), while the lyophilized water extract (C), contained some polar compounds, which might be anthraquinone glycosides.[96]

Timothy SY *et al.,* (2012) studied antifungal activity of aqueous and ethanolic leaf extracts of *Senna alata* Linn Is an important medicinal plant as well as ornamental flowering plant. The leaf decoction of *Senna alata* has been used to treat infectious diseases in north eastern Nigeria. This study was embarked upon so as to evaluate the safety and efficacy of Senna alata in the management of fungal infectious diseases. The leaves of the plant were collected, dried and extracted using water and 95% ethanol. The extracts were used for evaluating antifungal activity against five clinical isolates of pathogenic fungi. The result of this study showed a dose dependent antifungal activity of both aqueous and ethanolic leaf extracts on the five selected clinical isolates of pathogenic fungi. The extracts inhibited the growth of Candida albicans, Microsporium canis and Trichophyton mentagrophyte better than the ketoconazole 200 mg used as a positive control ($p<0.05$). The minimum inhibitory concentration of the water leaf extract of Senna alata for Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis and Trichophyton mentagrophyte were 26.90 mg, 32.40 mg, 29.50 mg, 30.30 mg and 27.80 mg respectively, while that of ethanol leaf extract of Senna alata for Candida albicans, Aspergillus niger, Penicillium notatum, Microsporium canis and Trichophyton mentagrophyte were 5.60 mg, 3.50 mg, 4.90 mg, 12.60 mg and 9.80 mg respectively. *Senna alata* has been found to exhibit a greater antifungal activity against some human pathogenic fungi and this has justified the traditional use of this plant in managing fungal diseases.[97]

Oke D. G *et al.,* (2018) Performed studies on the laxative properties of *Senna alata L.* and Hollandia yoghurt experiments on *Senna alata* aqueous extract and Hollandia yoghurt were done to determine active compounds responsible for their laxative properties and to further establish sample vulnerability to microbial attack. Phytochemical screening was performed on test substrates to analyse for alkaloids, flavonoids, tannins, Saponins, terpenoids and steroids. Escherichia coli (Gram negative), Micrococcus species (Gram positive), Klebsiella pneumoniae (Gram negative), Enterococcus species (Gram negative) and Salmonella species (Gram negative) were test bacteria while Penicillium species, Trichophyton species, Rhizopus species, Fusarium oxysporium and Aspergillus niger were test fungi used for microbial assays. The results show that alkaloids, tannins, Saponins, terpenoids and steroids were found in S. alata while only alkaloids, terpenoids and steroid were found in Hollandia yoghurt. S. alata had activity against Penicillium spp., Trichophyton spp. and Rhizopus spp., while Hollandia yoghurt showed no antifungal activity. Aqueous extract of S. alata and Hollandia yoghurt are however together active against test bacteria of which, K. pneumonia is common.[98]

Mansuang Wuthi-udomlert *et al.,* (2010) evaluated the antifungal activity of anthraquinone derivatives of *senna alata.* Leaf powder was used to obtain five extracts which contain anthraquinone compounds in different forms i.e. anthraquinone aglycones extract, anthraquinone glycoside extract, anthraquinone aglycones from glycosidic fraction, crude ethanol extract, and anthraquinone aglycones from crude ethanol extract. All extracts were tested against clinical strain of dermotophytes: Trichophyton rub rum, T. mentagrophyte, Epidermophyton floccosum, and Microsporium gypseum by diffusion and broth dilution techniques to find out the active form for antifungal activity. Thin layer chromatography was developed to demonstrate the fingerprints of chemical constituents of each extract. best in vitro antifungal activity of anthraquinone aglycones from glycosidic fraction qualitatively and quantitatively, compared to other extracts[99]

Funmilayo Adelowo *et al.,* (2017) reviewed of the phytochemical analysis of bioactive compounds in *Senna alata* the major problem man is facing is the growing rate of the activities of microbes. Virtually everything that surrounds man, even his environment are contaminated and polluted with these microbes. The use

of medicinal plants for the treatment of bacteria and fungi that caused related diseases gave rise to the introduction of antibiotics or natural drugs. The proper use of Senna alata as herbal medicine, their curative and therapeutic effects should be studied. It is of great importance to determine the chemical components (phytochemicals) and the ethno biological view of *Senna alata* extracts.[100]

U. Igweand FK *et al.,* (**2015),** investigated the leaf essential oil of *senna alata linn* and its antimicrobial activity The chemical constituents of the essential oil (extracted with isopropanol) of the leaves of Senna alata Linn were characterized using Gas Chromatography-Mass Spectrometry (GC/MS) technique and seven compounds were identified which include (6Z)-7,11-dimethyl-3-methylidenedodeca-1,6,10triene (2.42%), 4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene (3.80%), 4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one (2.91 %), 3,7-dimethylocta-1,6-diene (3.94%), hexadecanoic acid methyl ester (8.59%), hexadecanoic acid (3.31%) and octadecanoic acid methyl ester (75.03%). The extract exhibited marked antimicrobial activity against Staphylococcus aureus, streptococcus faecal is, Escherichia coli and Proteus mirabilis. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. The presence of these compounds in the leaves of S. alatamight is responsible for its antimicrobial activity as well as its use in the treatment of dermal diseases and other infections in herbal medicine in Nigeria.[101]

R.M. Kolawole *et al.,* (2015) Evaluated-MS analysis and antifungal activity of *Senna alata Linn* using standard agar well diffusion and broth dilution techniques, The different extracts showed reasonable zone of inhibitions but to varying degree of efficacies. Of the different extracts tested, ethanolic extract displayed the highest activity as reflected in their mean zone of inhibition ranging from 73.6mm to 167.4mm. This was followed by the activity of the chloroform extract that ranges from average zone of inhibition of 38-91mm. The aqueous extract showed the least mean zones of inhibition that ranges from 33-57mm. This also corroborated by the MIC and the MBC values. The GC-MS analysis of ethanolic extract led to identification of compounds including xylene, alcohol, aldehydes, alkanes, alkenes, fatty alcohol, acetic acid, ketones and ester. The compounds were identified by

comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Also, Senna alata Linn was also found containing Saponins, alkaloids, tannins, phlobatannins, Anthraquinones, cardenolides, steroidal ring and flavonoids. It can thus be inferred that Senna alata Linn possess good antifungal activity and such activities might be ascribed to the presence of the phytochemicals and some of the chemical constituents.[102]

S.Mohideen et al., (2005) investigated pharmacognosy of *Senna alata Linn*, is commonly known as Seemai Agathi in Tamil is well known for its various medicinal properties in Indian systems of medicine. Various parts of this plant are used as vermicide, astringent, purgative, expectorant and to treat skin diseases. The present work deals with the anatomy, quantitative microscopy, physical constants and fluorescence analysis of the plant leaves.[103]

Pharkphoom Panichayupakaranant et al, (2009) developed Quantitative HPLC determination and extraction of anthraquinone in Senna alata Leaves. A reversed-phase high-performance liquid chromatographic method the simultaneous determination of four Anthraquinones: rhein, aloe-emodin, emodin, and chrysophanol in Senna alata leaves. The method involves the use of a TSK-gel ODS80Tm column (5 µm, 4.6× ×150 mm) at 25°C with the mixture of methanol and 2% aqueous acetic acid (70:30, v/v) as the mobile phase and detection at 254 nm. The parameters of linearity, precision, accuracy, and specificity of the method were evaluated. The recovery of the method is 100.3–100.5%, and linearity (r2 > 0.9998) was obtained for all Anthraquinones. A high degree of specificity as well as repeatability and reproducibility (relative standard deviation values less than 5%) were also achieved. The solvent for extraction of Anthraquinones from S. alata leaves was examined in order to increase the anthraquinone content of the leaf extract. It was found that a solution of 5% hydrochloric acid (v/v), 5% ferric chloride (w/v), and 15% water in methanol (v/v) was capable of increasing the anthraquinone content in the leaf extract up to 1.67% (w/w).[104]

Simplice Joel Ndendoung tats m *et al.*, (2017) isolated the Antibacterial-guided isolation of constituents from Senna alata leaves with a particular reference against Multi-Drug-Resistant Vibrio cholera and Shigella Flexner Senna alata is widely used in Cameroon for the treatment of several infections which include

gonorrhoea, gastro-intestinal and skin diseases. Extraction of plant material was done with methanol, follow by partition with hexane. Separation and purification of compounds was done using a combination of chromatographic techniques. Isolated compounds were identified by means of spectroscopic methods and comparison with literature data. The antibacterial activity of extracts, fractions and compounds was assessed by evaluating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Multi-Drug-Resistant (MDR) Vibrio cholera and Shigella flexneri. Three secondary metabolites namely kaempferol, lute Olin and aloe emodin were isolated from methanol residue active extract. The antibacterial results showed that the Me OH residue extract, fractions A and C, as well as compounds 1-3 exhibited variables MIC values, depending on the bacterial strains. Aloe-emodin (MIC = 4 to 128 µg/mL) exhibited the highest antibacterial activity against MDR Vibrio cholera and Shigella flexneri.[105]

Olivier Tene Tcheghebe *et al.,* (2017) studied Ethno botanical uses phytochemical and pharmacological profiles, and toxicity of *Senna alata L*.ancient references for utilization of herbs, plants and other natural substances in clinical treatments. This knowledge is common among the people living in *Senna alata* is one of these plants largely used by traditional health practitioners with effectiveness. In the folkloric medicine, haemorrhoids, intestinal parasitosis, breast cancers. Pharmacological tests anti-cancer and antitumor, anti-inflammatory, hepatocurative, antibacterial and antifungal, bronchorelaxant and antiviral activities. Some bioactive co Anthraquinones, tannins, flavonoids, Saponins, alkaloids, steroids, triterpenoid, significantly present in the plant in traditional medicine. it has been scientifically proved that safe till a dose of 16875 mg/kg body weight. The literature on the ethno botanical uses, phytochemical and pharmacological profiles, as well as the toxicity of Senna alata .[106]

Dr.p.l.Rajagopal *et al.,* (2014) Review of Phytopharmacological Senna alata linn Senna alata (Guajava) is a beautiful flowering shrub that grows about 1 to 2 m in height. It produces pretty yellow flowers in a column that resemble yellow candlesticks earning its common name candlestick or candle bush. It is native to the Amazon Rainforest and can be found in Peru, Brazil, French Guiana, Guyana,

Suriname, Venezuela and Colombia. Due to its beauty, it has been cultivated around the world as an ornamental plant.[107]

CA Alalor *et al.,* (2012) investigated the antibacterial activity of the aqueous and methanol extracts of sundried leaves of Senna alata . Testing the extracts against some pathogenic Escherichia coli, staphylococcus aureus, bacillus subtilis, and pseudomonas aeruginosa using the agar cup plate method. The minimum inhibitory concentration (MIC) of the aqueous extracts against susceptible test organism was determined using the agar dilution method. The plant part can be used to treat infections caused by aureus and B subtilis which were susceptible. The Invitro findings justify the use of the extract of Senna alata in traditional medicine practice for the treatment of some external skin infections. Senna alata has shown to be a very versatile plant and can be a viable alternative as an antibacterial agent in the future either alone or in combination with other medicinal plants, if formulated into appropriate pharmaceutical dosage forms .It exists for in vivo research studies with preformulation testing, pharmaceutical dosage formulation and development, pharmacokinetics, safety and efficacy in patients.[108]

2.2. STUDY ON NIOSOMAL FORMULATION

Onochie *et al.,* (2013) formulated benzylpencillin niosomes by using thin film hydration technique. The encapsulation efficiency was found to be highest in batch A with value of 82.42 %. Batches B and C exhibited slow release, oral stability and good bioavailability in vivo. For in vitro and in vivo studies, batch B containing span 80, tween 65 and cholesterol was particularly stable and released its drug content in a controlled manner. The Cmax for the batches were higher than that of pure drug which has value of 55.04 mg/ml in vivo. The batches were high against the test microorganisms and exhibited antimicrobial activities greater than the unformulated drug against S.typhi, P.vulgaris and Ps. Aereuginosa.[109]

Katare O.P. *et al.,* (2001) prepared and in vitro evaluated the liposomal and niosomal delivery system for ant psoriatic drug Dithranol. The in vitro permeation study using mouse abdominal skin shows significantly enhanced permeation with vesicles as indicated by flux Dithranol from liposomes and niosomes as compared with the cream base. The entrapment of drug in vesicles reduces the dose and dose dependent side effect like irritation and staining.[110]

Jia - you. Fang *et al.,* (2001) studied the skin permeation and partitioning of antibacterial agent Enoxacin in liposomes and niosomes after tropical application. The in vitro study through mouse skin shows that the niosomal formulation as a higher stability after 48 hours incubation when compared to liposomes. The tropical liposomal and niosomal formulation of Enoxacin modulate drug delivery without significant toxicity.[111]

Anna M. fadda *et al.,* (2006) formulated and evaluated the in vitro cutaneous delivery of vesicle incorporated Tretinoin. The permeation study was carried out using both Tretinoin saturated and non-saturated vesicular formulations and also compared the results with liposomes formulation. The formulations which are saturated with tretinoin have shown to give higher cutaneous drug retention than both liposome and commercial formulation.[112]

Ibrahim. A. alsarra *et al.,* (2004) formulated proniosomes using different non-ionic surfactant and studied the in vitro release using rabbit skin. The in vitro results shows that proniosomes prepared with span 60 provided a higher Ketorolac flux across the when compared with tween 20 proniosomes. About 99% of drug was encapsulated in each formulation. The proniosomes was a promising carrier for the Ketorolac and other drugs especially due to their simple production and facile up.[113]

Yi.Hung Tsai *et al.,* (2000) formulated the proniosomes of Estradiol using different span surfactant and elevated the in vitro skin permeation from various proniosomes gel formulated across rat skin. Niosome suspension and proniosomes gel showed different behaviour in modulating transdermal delivery of estradiol across the skin.[114]

H.m shreedevi *et al.,* (2016) incorporated Stavudine into niosomes by using ether injection method. The physiochemical characterisation and in vitro permeation studies of the prepared vesicles by dialysis membrane to get the idea of drug release. The developed niosome formulation of Stavudine has shown great potential in the treatment of HIV by providing a prolonged release profile.[115]

Ahmad usama *et al.,* (2016) developed and characterized niosome-based delivery system of Meloxicam for in vitro performance. Niosome were prepared by reverse-phrase evaporation method. Results showed high encapsulation efficiency was obtained with ranged from about 81.93%. The highest entrapment efficiency was

obtained with 1:1 surfactant: cholesterol ratio and 15 mg drug loading, so niosomes prepared by this ratio were selected for further studies.[116]

Tamizharasi *et al.,* (2009) formulated and evaluated Gliclazide loaded niosomes to improve the oral bioavailability of the drug. The positive values of zeta potential indicated that the Gliclazide niosomes were stabilizing by electrostatic repulsive forces. The niosomes formulation could be a promising delivery system Gliclazide with improved bioavailability and prolonged drug release profile.[117]

P. Aravinth kumar *et al.,* (2013) studied Pregabalin in niosome for achieving prolonged release & longer duration of action. Niosomes containing Pregabalin was formulated using two surfactants such as span 40 & span 60 and evaluated for various parameters. Invitro release studies showed that the percentage amount of free drug release was 99.04% within 2.5 hours. Storage under refrigerated condition showed greater stability with 97.23% of drug at the end of 3 months.[118]

Ranga Priya *et al.,(*2013) developed niosomes of Zidovudine by ether injection method by varying ratios of span 80 and span 20 with cholesterol in ratio of 1:1,2:1 and 3:1and dicetyl phosphate added to the formulation to prevent aggregation of vesicle. The niosome with span and cholesterol in ratio of 2:1 had high drug release profile were found to be best formulation and they increase the therapeutic effectiveness of Zidovudine on prolong the therapeutic action of drug.[119]

Naresh ahuja *et al.,* (2008) prepared Lansoprazole niosome are by reverse phase evaporation method using homogenizers. Non-ionic surfactant based vesicles used as vesicles for drug formulation have been found to reduce the systemic toxicity of many drugs. Niosomes are characterized for its size range, entrapment efficiency and in –vitro release of drug.[120]

Alok kumar srinivastav *et al.,(*2014) formulated and evaluate Ofloxacin niosomes, it is a potent second generation flouroquinalone active against a broad range of gram positive and gram negative aerobic and anaerobic bacteria. The Ofloxacin niosomes were prepared by lipid film hydration method. The prepared Ofloxacin niosomes order with non-Fickian diffusion mechanism. Hence, the thin film hydration technique is an optimized technique for the preparation of niosomes.[121]

3.1. AIM OF THE WORK

- Skin is the most sensitive organ in the human body. Infection of the skin is caused by various pathogens such as bacteria, fungi and virus. Among the pathogens, fungi are the most causative organisms causing skin infections.
- Fungal infections represent an important paradigm in immunology, as they can result from either a lack of recognition by the immune system or over activation of the inflammatory response.
- However, plant based medicines are of interest in this context because they comprise safer or more effective substitutes for synthetically produced antimicrobial agents.
- Seemai Agathi also called as Vandukolli is the best medicinal plant against bacteria, fungi, virus and parasite.
- The botanical name of Seemai Agathi is *senna alata* which belongs to the family Caesalpiniaceae.
- This medicinal plant has got several uses including skin infections caused by bacteria, fungi etc.
- The study of antimicrobial activity of prepared niosome gel. It is against both bacterial, and fungal skin infection.
- Niosomes are one of the carrier mediated drug delivery system, so site specific drug delivery could be achieved.
- If it is given in niosome entrapped form, bioavailability of the drug could be improved. By entrapment of drug in niosomes, dose also could be reduced.
- Niosomes formed from self-assembly of hydrated synthetic nonionic surfactant monomers capable of entrapping variety of drugs. The size of these vesicles is in the nanometer range. This size range offers the decisive advantage of this class of pharmaceutical dosage forms as it allows drug targeting which often is not possible with free drug.

AIM OF THE WORK

- Senna Alalta .L niosomes are prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability.
- The aim of this work was using niosomal formulation could minimize the drug dose, reduce the toxic side effects and early recovery than the other conventional formulations of Senna Alalta .L. Hence Senna Alalta .L niosomal gel formulation was formulated and investigated for the feasibility of using niosome as a drug delivery system.
- The aim of the study was to investigate the feasibility of using niosome as a transdermal drug delivery system for Senna Alalta .L.
- Niosomes offer a versatile vesicle delivery concept with potential for delivering drug via transdermal route. This is possible when niosome is hydrated with water from skin on topical application under occulusive condition. Topically applied niosomes can increase residence time of drug in the stratum corneum and epidermis, while reducing the systemic absorption of the drug.
- It also improves the horny layer properties both by reducing transepidermal water loss and by increasing smoothness via replenishing lost skin lipids.
- Better targeting of drugs to the infected organs can be achieved by niosomal gel formulation due to the presence non-ionic surfactants with lipids. The presence of nonionic surfactants increase the permeability of Fluconazole through the biological membrane and also reduces the systemic toxicity of anti-infective drugs. Thus the therapeutic index of the Senna Alalta .L can be improved when given in niosomal gel formulation.

3.2. PLAN OF WORK

- Collection of leaf of *Senna alata leaves*
  - Selection of *Senna alata leaves*
  - Collection of the *Senna alata leaves*
  - Drying of the *Senna alata leaves*
  - Storage and Preservation of *Senna alata leaves*
  - Grinding of the *Senna alata leaves*

- Pharmacognosy of *senna alata leaves*
  - Macroscopical character
  - Microscopical character

- Extraction of *senna alata leaves*
  - Ethanolic extraction *senna alata leaves*
  - Drying of extract

- Phytochemical analysis of *senna alata leaves*

- Formulation of *senna alata* L.Niosome Gel

- Evaluation of senna alata L.Niosome Gel

- Invitro Evaluation of *senna alata* L.Niosome Gel
- Stability study of Niosomes
- Release kinetic studies
- Scanning Electron Microscopy of Senna Alata L.Niosomal gel
- Evaluation of Antimicrobial Activity of Senna Alata L.Niosomal gel formulation

4. MATERIALS AND METHODS

4.1 MATERIALS USED

MATERIALS	SOURCE
Senna Alata Linn. Leafs	: Ullaganeri mellur Main road Madurai
Cholesterol	: S.D.Fine Chem Ltd, Boisar
Potassium Dihydrogen Phosphate	: S.D.Fine Chem Ltd, Boisar
Disodium Hydrogen Phosphate	: S.D.Fine Chem Ltd, Boisar
Sodium Chloride	: S.D.Fine Chem Ltd, Boisar
Chloroform	: S.D.Fine Chem Ltd, Boisar
Methanol	: S.D.Fine Chem Ltd, Boisar
Sabourand Glucose Agar	: SRL CULTURE MEDIA PRODUCT
Agar Agar (Bacto)	: S.D.Fine Chem Ltd, Boisar
Carbopol 940	: S.D.Fine Chem Ltd, Boisar

4.2. INSTRUMENTS USED

1. Rotary Flash Evaporator : Equitron
2. Probe sonicator : Bandelin, Germany
3. Single Beam UV – Visible : 1201, Shimadzu Corporation,
4. Spectrophotometer Japan
5. Stability Chamber of 120 litres : Oswald, Mumbai

   Capacity
6. Zeta sizer : Malvern
7. Single pan electronic balance : Shimadzu Corporation,
8. Magnetic Stirrer : Remi
9. pH meter : ELICO LI 127
10. Hot Air Oven : Biochemi (Universal)
11. Autoclave : KEMI
12. 12.Laminar Air Flow Bench : Klenzaids, Mumbai

4.3. PLANT PROFILE

Botanical Name: ***Senna Alata Linn.***

Fig no: 3.*Senna alata L* Tree

SCIENTIFIC CLASSIFICATION [122]

- **Kingdom : Plantae**
- **Order : Fabales**
- **Family : Fabaceae**
- **Subfamily : Caesalpinioideae**
- **Tribe : Cassieae**
- **Sub tribe : Cassiinae**
- **Genus : Senna**
- **Species : S. Alata**

VERNACULAR NAME

Tamil: vandu koli Candle bush, Emperor Candle stick, Christmas candle, Acapulo, Ringworm bush and Calabra bush.

4.3.1. BOTONICAL DESCRIPTION

Senna alata is a shrub with usually an average height of between 1 and 5metres and has horizontally spread branches. Its leaves are par pinnate of between 30 to 60 cm long and consisting of 8 to 20 pairs of leaflets. Each leaflet is oblong or elliptic oblong and rounded at both ends. Its flowers are dense in auxiliary racemes, about 20 to50 cm long and 3 to 4 cm broad. The inflorescence looks like a yellow candle. The plant fruits are a thick, flattened with wings and glabrous pods. They grow well in full sun in a wide range of soils that retain moisture adequately. The species is easy to grow from the seed.

They can either be sown directly or started in a nursery and distributed all over the country up to 1,500 m above sea level; they are most often cultivated for medicinal purpose.[123]

Leaf

- Leaf Arrangement : Alternate Leaf
- Leaf Venation : Pinnate Leaf
- Leaf Persistence : Evergreen
- Leaf Type : Odd Pinnately compound
- Leaf Blade : 5 - 10 cm
- Leaf Shape : Oblong
- Leaf Margins : Entire
- Leaf Textures : Smooth, Coarse
- Leaf Scent : No Fragrance
- Color (growing season) : Green Color
- (Changing season) : Green

Flower

- Flower Showiness : True
- Flower Size Range : 10 – 20
- Flower Type : Spike Flower
- Sexuality : Monoecious (Bisexual)
- Flower Scent : No Fragrance
- Flower Color : Yellow, Orange

- Seasons : Summer, Fall Trunk Trunk
- Susceptibility to Breakage : Generally resists breakage
- Number of Trunks : Multi-Trunked Trunk Esthetical

Fruit

- Values : Smooth Fruit
- Fruit Type : Legume Fruit
- Showiness : False
- Fruit Size Range : 7 - 10
- Fruit Colours : Brown
- Seasons : Summer, Fall

4.3.2. Distribution:

It is a native to the Amazon Rainforest and can be found in Peru, Brazil, French Guiana, Guyana, Suriname, Venezuela and Colombia. Due to its beauty, it has been cultivated around the world as an ornamental plant and has naturalized in many tropical regions in the world including tropical Africa, tropical Asia, Australia, Mexico, the Caribbean islands, Melanesia, Polynesia, Hawaii and widely distributed throughout different parts of **India like Chhattisgarh, Maharashtra, West Bengal, Andhra Pradesh etc.**[124]

4.3.3. Worldwide Ethno medicinal Uses:

India- as an antidote, bactericide, diuretic, fungicide, insecticide, pesticide, purgative, Vermifuge, for asthma, bronchitis, constipation, dysentery, eczema, herpes, intestinal Parasites, rheumatism, skin disorders, snakebite, stomach ache, venereal diseases etc.

Africa- as an abortifacient, laxative, for parturition, scurvy.

Brazil- for anaemia, constipation, dermatitis, dyspepsia, fevers, hydropsy, liver Problems, menstrual disorders, skin problems, venereal disease, as a diuretic, Emmenagogues, laxative and as a purgative.

Cuba- as a diuretic, diaphoretic, laxative, against herpes, skin infections. Ghana- as an abortifacient, insecticide, purgative, vermifuge, for ascites,

Craw, eczema, gonorrhoea, herpes, leprosy, mycosis, parturition, Ringworm, shingles, skin problems, sores, wounds etc.

Haiti - as a depurative, diaphoretic, insecticide, tonic, vulnerary, for amygdalitis, Herpes, itch, measles, psoriasis, sore (throat), tonic, skin problems, prurigo, sores, Wounds etc**.**

Java- for herpes, itch, ringworm, scabies, syphilis, as a larvaecide etc.

Mexico- as a diaphoretic, diuretic, insecticide, purgative; for fever, rheumatism, Ringworms, skin infections, snakebite, syphilis etc.

Peru- as a diuretic, insecticide, laxative, vermifuge, for acaries, hepatitis, herpes, Intestinal parasites, ringworm, skin problems, snakebite, urinary infections etc.

Samoa - as a purgative, for ringworms, skin problems, snakebite etc.

Trinidad - as a bactericide, laxative, vermifuge, for diarrhoea, eczema, herpes, venereal Diseases, vitiligo etc.

Venezuela- as a diuretic; for itch, skin problems

Elsewhere- as an antidote, bactericide, diuretic, fungicide, insecticide, pesticide, Purgative, vermifuge, for asthma, bronchitis, constipation, dysentery, eczema, herpes, Intestinal parasites, rheumatism, skin disorders, snakebite, stomach ache, venereal Diseases etc.

LEAF

Fig no: 4. *Senna Alata L.* Leaf

4.3.4. PHYTOCHEMICALS PRESENT IN SENNA ALATA LEAFS

Senna alata L. is known to contain an array of bioactive components or pharmacologically active compounds known as phytochemicals. Several researchers have identified different phytochemicals. There are many compounds present in the plant phenolic compounds, alkaloids, anthraquinone, tannins, steroids, flavonoids among others

Phytochemical Analysis the freshly prepared extracts were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, glycosides, Saponins and phenols.

4.3.5. PHYTOCHEMICAL ANALYSIS

Test for Alkaloides:

To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids.[123]

Test for Tannins:

Two drops of 5% FeCl3 was added to 1ml of the plant extract. The appearance of a dirty green precipitate indicated the presence of tannins

Test for Flavonoids:

To 1ml of the extract was added 3 drops of ammonia solution (NH3+) followed by 0.5ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids

Test for Steroids:

To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (vi) acid (H2SO4). A red colouration confirmed the presence of steroids

Test for Resins:

To 5ml of the extract was added 5ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins.

4.3.6. Traditional uses

S.alata L. leaves and flowers have long been traditionally used as laxative and antifungal agents

Use as laxative

1. Eight leaves are sun dried and powder an infusion is made from the powdered leaves and taken before bed time
2. A few flowers cooked in boiling water and taken with the a special sauce called **Namprik**
3. Three to five branches with leaves are boiled with water (1500 ml). The decoction is boiled until about one third of water used is obtained. Salt is added to the infusion to give it a salty taste. One glass of the decoction is taken.

Treatment of ringworm or Tinea versicolor

1. Fresh leaves are pounded and the juice obtained is applied over the infected area.
2. Three to four fresh leaves are pounded. Lemon juice is then added. The obtained juice applied over the infected area.

4.3.7. ETHNOMEDICAL USES

Stems

Treatment of yaws, ringworm, Tinea versicolor, constipation, urinary stone, anthelmintic and cardio tonic

Leaves

Treatment of skin diseases, urinary stone, ringworm, Tinea versicolor, laxative, cardio tonic, and Expectorant.

Flowers

Laxatives and improvement of appearance and texture of skin

Pod

As an anthelmintic

Seed

Treatment of skin diseases, constipation and as an anthelmintic**.**

Whole plant

As an anthelmintic and antipyretic

Not specific part

Treatment of skin diseases, haemorrhoids, chronic gastrointestinal aliments of children between the ages of 5 and 13 years characterized by marked malnutrition, usually associated with intestinal parasitism.

4.3.8. CHEMICAL SUBSTANCE

Plant part:

Leaves

- **Flavonoid**
- **Glycoside**
- **Anthraquinone**
- **Anthraquinone glycoside**
- **Polyphenol**

Root

- Anthraquinone

Stem

- Flavonoid
- Glycoside
- Anthraquinone
- Anthrone
- Sterol

Fruit

- Anthraquinone

Seed

- Polyalcohol
- Carbohydrate
- Flavonoid
- Glycoside
- Sterol

4.4. EXCIPIENT PROFILE

4.4.1 CARBOPOL

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are primary produced from polymer having particles of about 0.2 to 6.0 micron in diameter. Carbomer-940 is to be a polymer of acrylic acid cross-linked with alkyl-sucrose: CH = CHCH2-O-sucrose.Carbomer-940P is the pharmaceutical grade of Carbomer-940.[125]

Molecular weights for Carbomers - 940:

Approx. 500,000 to 4,000,000.g

Colour: White, light, acidic, hygroscopic powder.

Particle size:

Flocculated powder having a median diameter of 2 to 7 microns.

Solubility / swelling properties:

They are insoluble due to their cross linked nature and high molecular weight. They get swell in water and some polar solvents, producing viscous dispersions.

Topical Applications:

Carbopol 940 is very well suited to aqueous formulations of the topical dosage forms such as hydrogel. Many commercial topical products available today have been formulated with these polymers. They provide the following plentiful advantage to topical formulations[126]

Safe & Effective

Carbopol polymers have a long antiquity of safe and effective use in topical gels, creams and ointments. They are also supported by board toxicology studies

Non Sensitizing

Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage.

Biocompatible

Carbopol polymers provide a magnificent vehicle for drug delivery. Due to their high molecular weight, they are not able to penetrate the skin or affect the therapeutic efficacy of the drug. Superior thickening, suspending, & emulsification properties for Topical preparations.

Plant Profile

Products with a wide range of viscosities and flow properties have been successfully formulated and commercialized.

Advantages with use of Carbopol gels as a vehicle

Good rheological properties on the application site.[127]

- Substitute to oil-based ointment formulations
- Viscosity is high at low concentration
- Compatibility
- Better bio adhesive properties
- Good thermal stability

Uses:

In dermatological formulation Carbopol used as excellent vehicle. They can be used in condition when controlled pattern is desired in drug delivery system. These polymers are anionic polymers that need naturalization to become jellified.

4.4.2. SODIUM CARBOXYMETHYL CELLULOSE

Synonyms: [128]

Sodium cellulose glycolate, Na CMC, CMC, cellulose gum, sodium CMC; INS No. 466

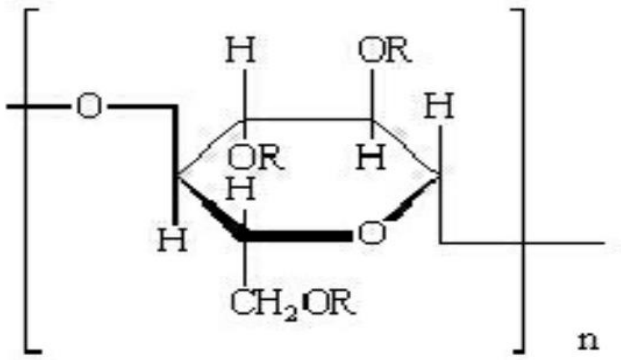
Chemical names:

Sodium salt of carboxymethyl ether of cellulose

Chemical formula:

[C6H7O2 (OH)x(OCH2COONa)y]n where n is the degree of polymerization x = 1.50 to 2.80 y = 0.2 to 1.50 x + y = 3.0 (y = degree of substitution)

Structural formula



Where R = H or CH2COONa

Formula weight

Structural unit with a degree of substitution of 0.20: 178.14

Structural unit with a degree of substitution of 1.50: 282.18

Macromolecules: greater than about 17,000 (n about 100) [129]

Description

White or slightly yellowish, almost odourless hygroscopic granules, powder or fine fibres

FUNCTIONAL USES

Thickening agent, stabilizer, suspending agent

EXPERIMENTAL INVESTIGATION

5. EXPERIMENTAL INVESTIGATION

5.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Collection and Identification of the plants

The fresh leaves of *Senna alata L*. was collected in the November to February month of from Ullaganeri mellur main road Madurai. The plant was identified and authenticated by Dr.D.Stephen, Assistant professor, Department of Botany The American College, Madurai.

Drying of the plant part

The collected plants were washed with water and unwanted materials were discarded. Collected plants were air and sun dried for 14 days.

Storage and Preservation of plant part

Most plant parts from desired plants have undergone a period of storage before they were finally used for research purpose in the laboratory. During this period many undesirable changes may occur in the plant parts if they were not properly stored and preserved against the reabsorption of moisture, oxidation, excessive heat, humidity, direct sunlight, growth of molds and bacteria and infestation by insects and rodents. Proper storage and preservation of plant parts are thus are very important factors in maintaining a high degree of quality in them. All efforts towards proper storage should be geared to protect the drugs from all the above deteriorating factors and agents.

Grinding of the plant parts

The Fresh leaves were air dried and then dried in a hot air oven at 50^{o} C for 5 hours. The dried leaves were ground with an electronic mill and passed through a sieve (20 mesh).

The fresh leaves was used for the study of morphological and microscopically characters; Whereas the dried leaves powder was used for determination of powder microscopy, physicochemical characterization and phytochemical analysis.

5.2.1 MACROSCOPIC CHARACTERS

The leaves of *Senna alata L*. was subjected to microscopic studies which comprised of organoleptic characteristics viz. colour, odour, appearance,taste,shape texture,fracture ,etc. of the drug This parameters are considered as quite useful in quality control of the crude drug and were evaluated as per standard WHO guidelines

The observation has been tabulated in the following table

Table No : 1

Leaflet shape	oblong; obovate
Leaf odour	characteristic odour
Leaf taste	Bitterish unpleasant taste
Leaf colour	Green
Texture	Coarse
Leaflet margin	Entire

The fresh leafs is green colour, Leaf arrangement: alternate , Leaf type: even Pinnately compound, Leaflet margin: entire, Leaflet shape: oblong; obovate, Leaflet venation: pinnate, Leaf type and persistence: evergreen, Leaflet blade length: 2 to 4 inches, Leaf colour: green . The dried leaf is light green colour

5.2.2. MICROSCOPIC CHARACTERISTICS OF *SENNA ALATA* LEAF

Fresh leaves of *Senna Alata L.* 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 leaf were made and examined microscopically. Histochemcal reactions were applied with staining reagents on transverse sections and on leaves powder by reported methods. Photomicrographs of the microscopically sections were taken with the help of Digital microscope,

5.2.3. PHYSICOCHEMICAL EVALUATION OF *SENNA ALATA* LEAF

Analysis of Physicochemical constants of the powder leaves has been done to evaluate the quality and purity of the drug. Various Physicochemical parameters like Moisture contents, foreign organic matters. Ash Values and Extract Values were calculated as per WHO Guidelines. The information collected from these test was useful for standardization and obtaining the quality standards.

5.2.4. EXTRACTION PROCESS OF *SENNA ALATA* LEAF

SOXHLET EXTRACTION

The powdered leaves of Senna alata L. (10.0 g) were extracted with 300 ml of 80% ethanol in a soxhlet apparatus. The extraction was continued until the extraction was exhausted. Each extract was then combined, filtered and evaporated to dryness on a hot water bath to yield a soxhlet crude extract (2.56 g). The extraction method which promoted the extract containing the maximum content of total Anthraquinones would be choosen as the appropriate method for further extracting the leaf samples of Senna alata L. Collected from various locations.

Drying of extract:

Using rotary evaporator, the methanolic extract of plant was evaporated at 55-60 degree Celsius temperature and a rotation speed of 160-180 rpm for 1 month. After this drying process, a slurry concentration were obtained, which were kept in small 50 ml beakers for further drying. During transfer to the beaker the extracts were rinsed by acetone.

5.2.5. PHYTOCHEMICAL INVESTIGATIONS OF *SENNA ALATA* LEAF

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

Determination of absorbance maximum (λmax) [130]

Senna alata L .was dissolved in phosphate buffer saline pH 7.4 solutions with 20 μg/ml concentration was prepared by suitable dilution.

Senna alata 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 298 nm. The drug was later quantified by measuring the absorbance at 240 nm in phosphate buffer saline pH7.4.

Standard curve for *Senna alata L* (by UV method)

Isolation of emodin

Ethanol plant fraction was applied on precoated silica gel G plate and run in solvent system chloroform and methanol (80:20) shown three different distinct spots. Then ethyl acetate fraction was subjected for column chromatography using silica gel G and Chloroform and methanol (80:20) as a mobile phase. Compound flavonoid was isolated.

Preparation of primary stock solution

Senna alata L 100 mg was weighed and dissolved in phosphate buffer saline pH7.4 in a 100 ml volumetric flask. The flask was shaken and volume was made up to the 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 2ml and placed into 100ml volumetric flask. The volumetric was made up to mark with phosphate buffer saline pH 7.4 give a stock solution containing 20μg/ml.

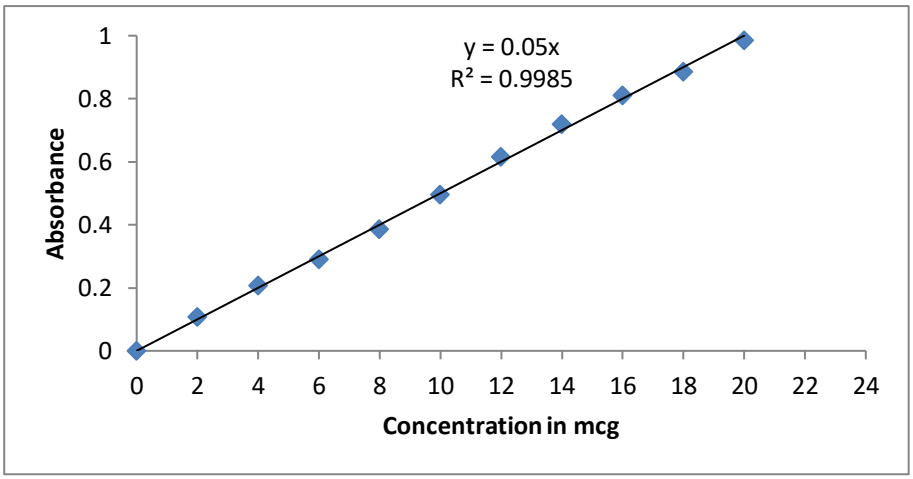
Preparation of sample solution

Appropriate volumes of aliquots (1to10ml) from standard *Senna alata 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 pH7.4 to obtain concentration of 10,20,30,40,50,60,70,80,90,and 100μg/ml. Absorbance of each solution against phosphate buffer saline pH7.4 as blank were measured at 298 nm and the graph of absorbance against concentration were plotted shown fig no:

Table No. 2 Standard curve data *Senna alata L.* ethanolic extract (By U.V method)

Concentration in µg/ml	Absorbance at 298 nm
10	0.109
20	0.208
30	0.291
40	0.387
50	0.496
60	0.617
70	0.721
80	0.812
90	0.886
100	0.987

Fig no : 5



5.2.6. FORMULATION OF *SENNA ALATA LINN* NIOSOME

Niosome was prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform: methanol mixture (1:1 v/v) in 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60^{o}C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60^0 C ± 2^0 C for a period of 1hour until the formation of niosomes. The batches were subjected to sonication process for 2 min using probe sonicator.

The gel formulation was prepared by incorporating the optimized formulation into a suitable gel base. The gel base selected for incorporation of niosomes with carbopol, SCMC base.

Gel Formula with carbopol, scmc, base Ingredients

Senna alata linn

Carbopol 940, SCMC

Glycerine

Distilled Water

Table No. 3 COMPOSITION OF SENNA ALATA L.NIOSOMES

S.No	Formulation code	SENNA ALATA.L In mg	cholesterol	SCMC	Carbopol
1	F1	100	50mg	2%	_
2	F2	100	50mg	3%	_
3	F3	100	50mg	4%	_
4	F4	100	50mg	_	0.5%
5	F5	100	50mg	_	0.75%
6	F6	100	50mg	_	1%
7	F7	100	50mg	_	1.25%

5.2.7. EVALUATION OF SENNA ALATA L. NIOSOMES

DRUG & POLYMER COMPATABILITY STUDIES BY FTIR:

Pressed pellet technique was used to handle the sample in FTIR spectrometer. In this technique a pinch of sample was mixed with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted in to special holder of IR spectrometer.

IR spectrums for pure drug alone and physical mixture of drug and polymers are taken. The spectrum of physical mixture was compare with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture, hence there was to no significant interaction between drug and excipients

Removal of unentrapped drug from niosome

The unentrapped drug from niosome was removed by dialysis method. Niosome suspension was placed in 3cm × 8cm long dialysis bag whose molecular weight cut off was 12,000. The dialysis bag was then placed in 250 ml beaker containing phosphate buffer saline of pH 7.4 with constant stirring by means of a magnetic stirrer. Dialysis was carried out for 24 hour by replacing the buffer with fresh for every 3 hours.

Size Analysis

By optical microscopy

A drop of niosome suspension was placed on a glass slide and it was diluted. A cover slip was placed over the diluted niosome suspension and evaluated the average vesicle size and shape by an ordinary optical microscope using a recalibrated ocular eye piece micro meter.

By scanning electron microscopy

The optimized formulation was morphologically characterized by scanning electron microscopy. The sample for SEM analysis was mounted on the specimen stub was placed on to a carbon coated grid to leave a thin film before drying of this film on the grid. It was negatively stained with 1% of phosphor tungstic acid (PTA).

For this ,a drop of staining solution was added on to the film and the excess of the solution was drained of the filter paper. The grid was allowed to air dry thoroughly and was then visualized using a scanning electron microscope with an accelerating voltage of 80kv.

Percentage encapsulation of drug

Vesicles containing senna alata L were separated from encapsulated drug by dialysis. Senna alata linn Niosomal preparation of 0.5 ml was taken after dialysis. To this 0.5ml of 10% triton X- 100 was added and incubated for 1hour .The triton X-100 was added to lyse the vesicles in order to release the encapsulated senna alata linn. Then it was diluted with phosphate buffer saline solution (pH 7.4) and filtered through whatmann filter buffer and triton X-100 mixture as blank. From the absorbance value, the concentration of drug in mcg/ml was found using the standard curve

$$\text{Percentage drug loading (PDL)} = \frac{\text{Amount of drug entrapped (mg)}}{\text{Total amount of drug added (mg)}} \times 100$$

5.2.8. *IN VITRO* CHARACTERISATION

Niosomal preparation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 200 ml of diffusion medium (Phosphate buffer saline pH 7.4). The medium was maintained at a temperature of 37 ±0.5 C. It was stirred by means of magnetic stirrer at a constant speed. Sample of 1ml (diffusion medium) was withdrawn at every 24 hours and replaced the diffusion medium, so that the volume of diffused medium was maintained constant at 200ml.The samples were measured spectrophotometrically at 266nm. The release was compared with a marketed senna alata L gel.

5.2.9. STABILITY STUDIES OF *SENNA ALATA* L NIOSOMES

The optimized formulation F6 was subjected to stability study for one month at 4^0 C (refrigerator), room temperature and at 45^0C/75% RH. At the interval of 30 days, samples of niosomal formulation were taken and evaluated for the entrapment efficiency and in vitro release of drug Entrapment efficiency of optimized formulation F6 kept at 4^0C 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 at room temperature and at 45^0C/75%RH were 92% and 81% respectively after 30 days of stability study

5.3. RELEASE KINETICS STUDIES

For estimation of the kinetic and mechanism of drug release, *in vitro* drug release study of niosomes 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.

b. 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.

c. **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.

d. 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.

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

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,

$$\text{Log } Mt / M\alpha = \text{Log } K + n \text{ Log } t$$

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

Table No: 4 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

5.4. Morphology

5.4.1. Binocular microscopy and Scanning Electron Microscopy (SEM)

The niosomes morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscopy (SEM) at different magnifications. A few mg of prepared niosomes was gold coated using a Hitachi HVSJGB Vacuum evaporator. Coated samples were viewed and photographed in a Hitachi S-450 SEM operated at 20 k v

5.5. Evaluation of Antimicrobial Activity of Senna Alata L.niosomal gel formulation

The antimicrobial activity of senna alata L niosomal formulation was carried out by agar plate method with test organism.

Materials required

Organism used

- candida albicans, spores
- *Aspergillus niger*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa,*
- *Escherichia coli*

Standard used

Fluconazole, ciprofloxin

Medium used

MGYP medium

Nutrient Agar medium

Potato Dextrose Agar medium MGYP medium

Medium used for broth culture

MGYP broth

Nutrient Agar broth

Potato Dextrose Agar broth

Requirements

- Sterile buds
- Sterile boiling tubes having 27 ml water
- Sterile tarsons microtips of (2- 200µl) and (200-1000µl)
- Micropipettes of 1 ml and 50µl petri plats of 9cm diameter sterilized in a hot air oven 160 C for 1 hr.

Media composition:

Table No. 5 MGYP medium: (pH: 6.4-6.8)

S.No	Ingredient	Quantity taken
1.	Malt extract	0.6 g
2.	Glucose	2.0 g
3.	Yeast extract	0.6 g
4.	Peptone	1.0 g
5.	Distilled water	200.0 ml
6.	Agar	4.0 g

MGYP medium:

- The respective quantity of Malt extract, Glucose, Yeast extract, Peptone, Agar were added in 200ml of distilled water.
- Heat to boiling to dissolve the medium completely
- Then sterilized by autoclaving 15 lb at 121^0C for 15 min.

  The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification. 15µl of inoculums of the bacteria was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each

of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 37^0C ±1^0C for 24 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Table No. 6 Nutrient Agar medium: (pH: 7-7.5)

S.No	Ingredient	Quantity taken
1.	Beef extract	2.0 g
2.	NaCl	1.0 g
3.	Peptone	2.0 g
4.	Distilled water	200.0 ml
5.	Agar	4.0 g

Nutrient Agar medium:

- The respective quantity of Beef extract, NaCl, Peptone, Agar adds in 200ml of distilled water.
- Heat to boiling to dissolve the medium completely
- Then sterilized by autoclaving 15 lb at 121^0C for 15 min.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification. 15µl of inoculums of the bacteria was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 37^0C ±1^0C for 24 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Table No.7 Potato Dextrose Agar medium: (pH: 6-7)

S.No	Ingredient	Quantity taken
1.	Pieces of potato	40.0 g
2.	Dextrose	4.0 g
3.	Yeast extract	0.02 g
4.	Distilled water	200.0 ml
5.	Agar	4.0 g

Potato Dextrose Agar medium:

- 40.0 g of peeled potatoes are cut into small pieces and suspended in 200.0 ml of distilled water.
- Steamed for 30 min. decant the extract or filter through muslin cloth and make the final volume to 200.0 ml.
- Add 4.0 g of dextrose, 0.02 g of yeast extract and 4.0 g of agar.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification.15µl of inoculums of the bacteria and fungi was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 28^0C for 48 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

RESULTS AND DISCUSSION

6. RESULTS AND DISCUSSION

6.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Collection and identification of plant

The fresh leaves of Senna alata was collected in the November to February month of from Ullaganeri mellur main road Madurai. The plant was identified and authenticated by Dr.D.Stephen, Assistant professor, Department of Botany The American College, Madurai.

Drying of the plant part

The collected plants were washed with water and unwanted materials were discarded. Collected plants were air and sun dried for 14 days.

Fig. No.6 Dried *Senna alata* leaves

Storage and Preservation of plant part

Most plant parts from desired plants have undergone a period of storage before they were finally used for research purpose in the laboratory. During this period many undesirable changes may occur in the plant parts if they were not

properly stored and preserved against the reabsorption of moisture, oxidation, excessive heat, humidity, direct sunlight, growth of molds and bacteria and infestation by insects and rodents. Proper storage and preservation of plant parts are thus are very important factors in maintaining a high degree of quality in them. All efforts towards proper storage should be geared to protect the drugs from all the above deteriorating factors and agents.

Grinding of the plant parts

The dried small pieces of plant parts were grinded into small fine particles by a grinder machine from. The powder was stored in an air tight container and kept in a cool dark and place until analysis commenced The dried plant powder as identified and authenticated by Dr.D.Stephen, Assistant professor, Department of Botany The American College, Madurai.

6.2.1 MACROSCOPIC CHARACTERS OF *SENNA ALATA* LEAF

Senna alata is a shrub with usually an average height of between 1 and 5metres and has horizontally spread branches. Its leaves are par pinnate of between 30 to 60 cm long and consisting of 8 to 20 pairs of leaflets. Each leaflet is oblong or elliptic oblong and rounded at both ends. Its flowers are dense in auxiliary racemes, about 20 to50 cm long and 3 to 4 cm broad. The inflorescence looks like a yellow candle. The plant fruits are a thick, flattened with wings and glorious pods. They grow well in full sun in a wide range of soils that retain moisture adequately. The species is easy to grow from the seed.

They can either be sown directly or started in a nursery and distributed all over the country up to 1,500 m above sea level; they are most often cultivated for medicinal purposes

Fig no: 7 *senna alata L*

Organoleptic character

- **Leaf odour : characteristic odour**
- **Leaf taste : Bitterish unpleasant taste**
- **Leaf colour : Dark green**
- **Leaflet margin : entire**
- **Leaf pairs : 10-12 pairs of leaflets**
- **Leaflet shape : oblong; obovate**

Quantitative Morphology

- **Leaflets Length : 7.2-11.2 cm in length**
- **Leaf lets breath : 3.1-8.4 cm in breadth**

6.2.2. MICROSCOPIC CHARACTERISTICS

Powder Microscopy of leaf

The powder preparation leaf shows the following inclusion:

Fig no: **Powder Microscopy of leaf**

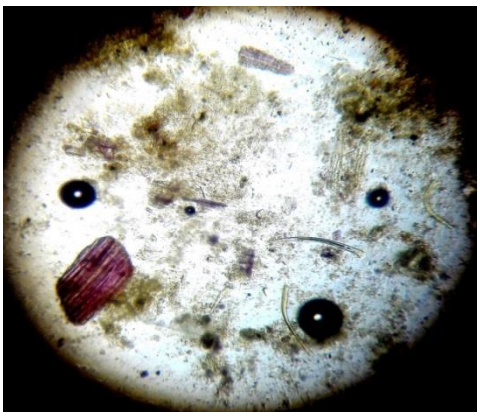


Fig No: 8

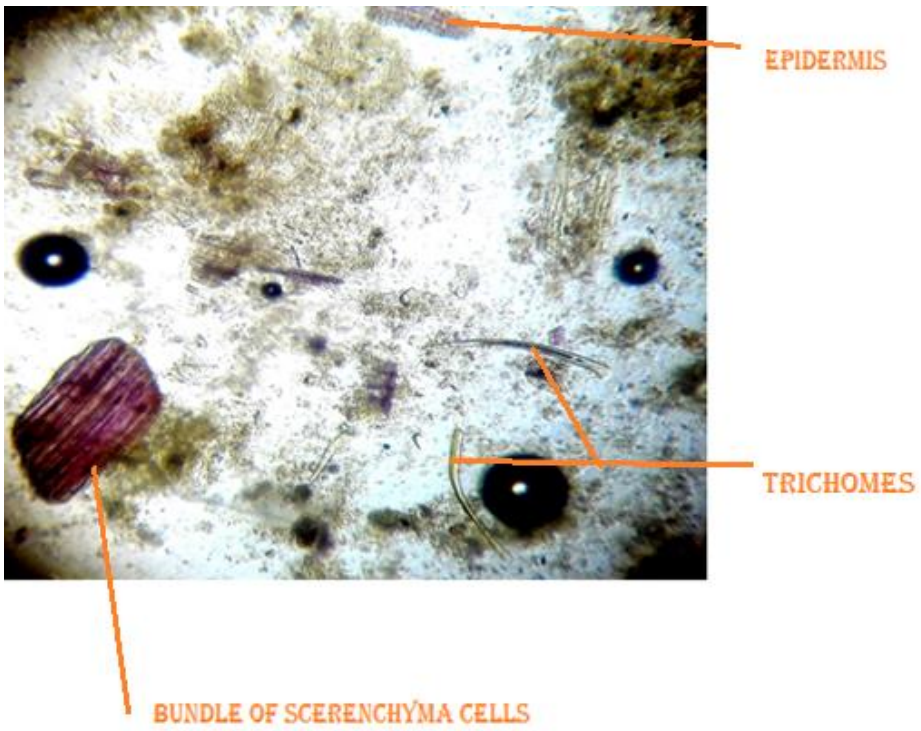


Fig No: 9 Powder microscopy

6.2.3. PHYSICOCHEMICAL EVALUATION OF *SENNA ALATA* LEAF

Ash Values: Air dried powdered samples of the whole plants of Cassia alata was investigated for total ash, acid insoluble ash and water-soluble ash values by following procedure.

Total Ash Value:

About 2gm of the Cassia alata was weighed accurately and spread as a fine layer at the bottom of a tarred silica crucible. The crucible was incinerated at 500 – 600 ºC in a muffle furnace until free from carbon. Then the crucible was cooled & weighed, the percentage total ash content was calculated.

Acid Insoluble Ash Value:

The ash obtained from the total ash was boiled with 25ml of HCl for 5 min. The insoluble ash was collected on ash less filter paper, washed with hot water. The filter paper along with the residue was transferred to a tarred silica crucible and ignited at a temperature not exceeding 600 ºC to constant weight. The percentage of the acid insoluble ash was calculated.

Water-Soluble Ash Value:

The total ash obtained was boiled with 25ml of water for 5 min. The insoluble matter is collected on an ash-less filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450ºC. The weights of insoluble matter were subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water-soluble ash was calculated.

Extractive Values

Chloroform Soluble Extractive Value:

15gm of coarsely powdered air-dried cassia alata was accurately weighed, in a glass-stopper conical flask. 100 ml of chloroform was added shaken vigorously and vigorously intermediately for 24 hours and reweighed the flask. The flask evaporated, cooled and weighted

Alcohol soluble extractive value

15gm of coarsely powdered air-dried cassia alata was accurately weighed, in a glass-stopper conical flask. 100 ml of alcohol (90%v/v) is added shaken vigorously intermediately for 24 hours and reweighed the flask. The flask evaporated, cooled and weighted.

Physical constants

Ash Values

- **i) Total ash – 7.84%**
- **ii) Acid – insoluble ash-Nil**
- **iii) Water soluble ash – 7.59%**

Extractive values

- **Chloroform – 7.4%**
- **Alcohol – 5.8 %**

6.2.4. EXTRACTION PROCESS

SOXHLET EXTRACTION

The powdered leaves of S. alata (10.0 g) were extracted with 300 ml of 80% ethanol in a soxhlet apparatus. The extraction was continued until the extraction was exhausted. Each extract was then combined, filtered and evaporated to dryness on a hot water bath to yield a soxhlet crude extract (2.56 g). The extraction method which promoted the extract containing the maximum content of total Anthraquinones would be choosen as the appropriate method for further extracting the leaf samples of S. alata collected from various locations.

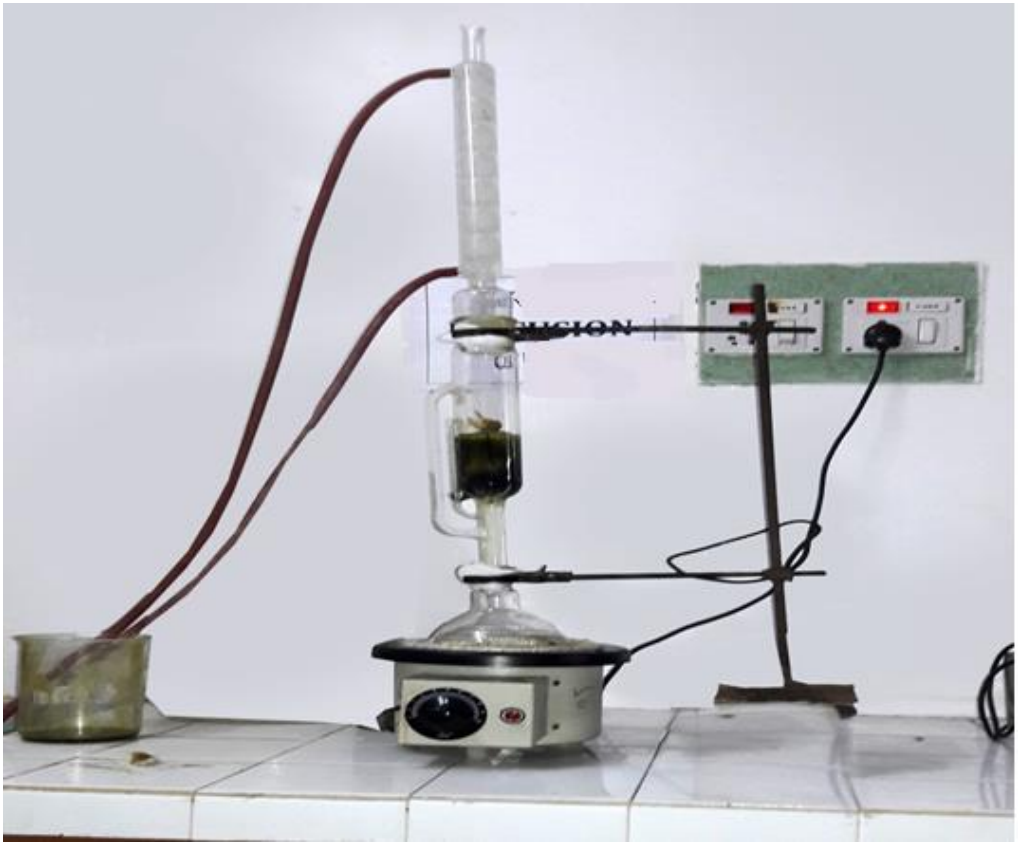


Fig No: 10 soxhlet apparatus

Drying of extract:

Using rotary evaporator, the methanolic extract of plant was evaporated at 55-60 degree Celsius temperature and a rotation speed of 160-180 rpm for 1 month. After this drying process, a slurry concentration were obtained, which were kept in small 50 ml beakers for further drying. During transfer to the beaker the extracts were rinsed by acetone.

6.2.5. PHYTOCHEMICAL INVESTIGATIONS *SENNA ALATA* LEAF

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

Phytochemicals Present in Senna alata

- Senna alata is known to contain an array of bioactive components or pharmacologically active compounds known as phytochemicals.

- Several researchers have identified different phytochemicals. There are many compounds present in the plant phenolic compounds, alkaloids, anthraquinone, tannins, steroids, flavonoids among others

Phytochemical Analysis of Senna alata

- Phytochemical screening is done to ascertain the scientific assessment of the claim of the therapeutic potency.
- The healing potency of these phytochemicals have taken a new dimension since there have been recent interest by the Scientists on what constitutes each medicinal plants.
- There have been different measures or steps taken for the analysis of these phytochemicals.
- Senna alata are reported to contain a variety of secondary or bioactive compounds, known as phytochemicals.

- They screened the leaves extracts for its phytochemicals, antibacterial and antifungal activities. The methanolic extract showed the highest activity than the ethanolic and petroleum ether extracts
- This may be due to the effect of polarity of the solvent. The unidentified active components purified from preparative thin layer chromatography exhibited low activities against Mucor, Rhizopus and Aspergillus Niger at concentration of 70µg/ml while higher activity was exhibited against all the test bacteria and fungi at 860µg/ml.
- Senna alata leaf decoction has been used to treat infectious diseases in north eastern Nigeria. Timothy etal. Screened the leaves extract for their activity against infectious diseases.
- In the attempt to perform this, the leaves of the plant were collected, dried and extracted using water and 95% ethanol and evaluated against five clinical isolates of pathogenic fungi for their antifungal activity.

Table No: 8 Phytochemical analysis

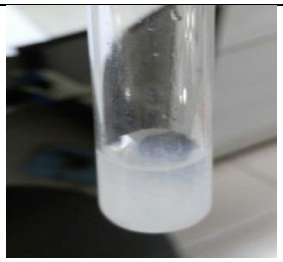
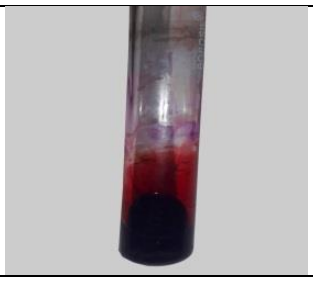
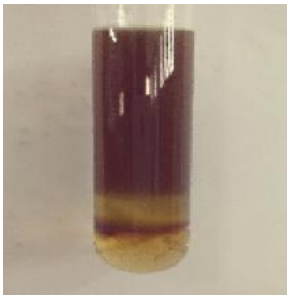
TEST	OBSERVATION	RESULT
Test for Alkaloides: To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer's reagent	The appearance of a creamy white precipitate confirmed the presence of alkaloids	
Test for Tannins: Two drops of 5% FeCl3 was added to 1ml of the plant extract	The appearance of a dirty green precipitate indicated the presence of tannins	
Test for Flavonoids: To 1ml of the extract was added 3 drops of ammonia solution (NH3+) followed by .5ml of concentrated HCl	The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids	
Test for Steroids: To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (vi) acid (H2SO4).	A red colouration confirmed the presence of steroids.	
Test For Glycosides Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution.	The upper aqueous layer becomes pink	

Table No.9 Results of Phytochemical Analysis of Senna Alata L. Extracts.

NATURE	ETHANOLIC EXTRACTION
Alkaloids	+
Tannins	+
Flavonoids	+
Steroids	+
Glycosides	+

6.2.6. FORMULATION OF SENNA ALATA LINN NIOSOME

Niosome was prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform: methanol mixture (1:1 v/v) in 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60 C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60^0 C ± 2^0 C for a period of 1hour until the formation of niosomes. The batches were subjected to sonication process for 2 min using probe sonicator.

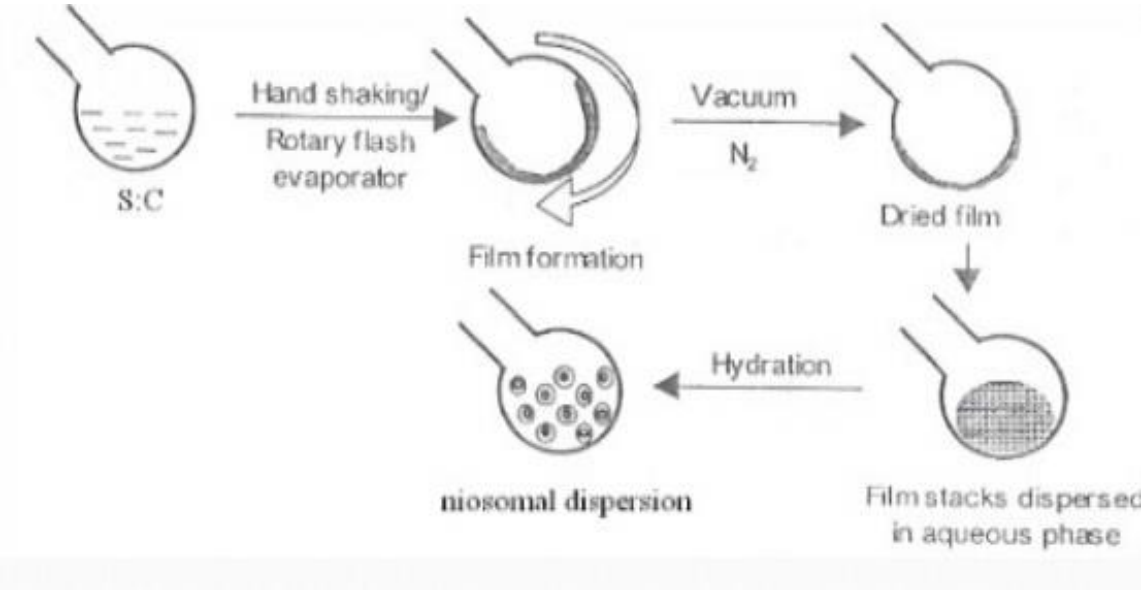


Fig No ; 11 Thin film hydration method

6.2.7. EVALUATION OF SENNA ALATA L. NIOSOMES

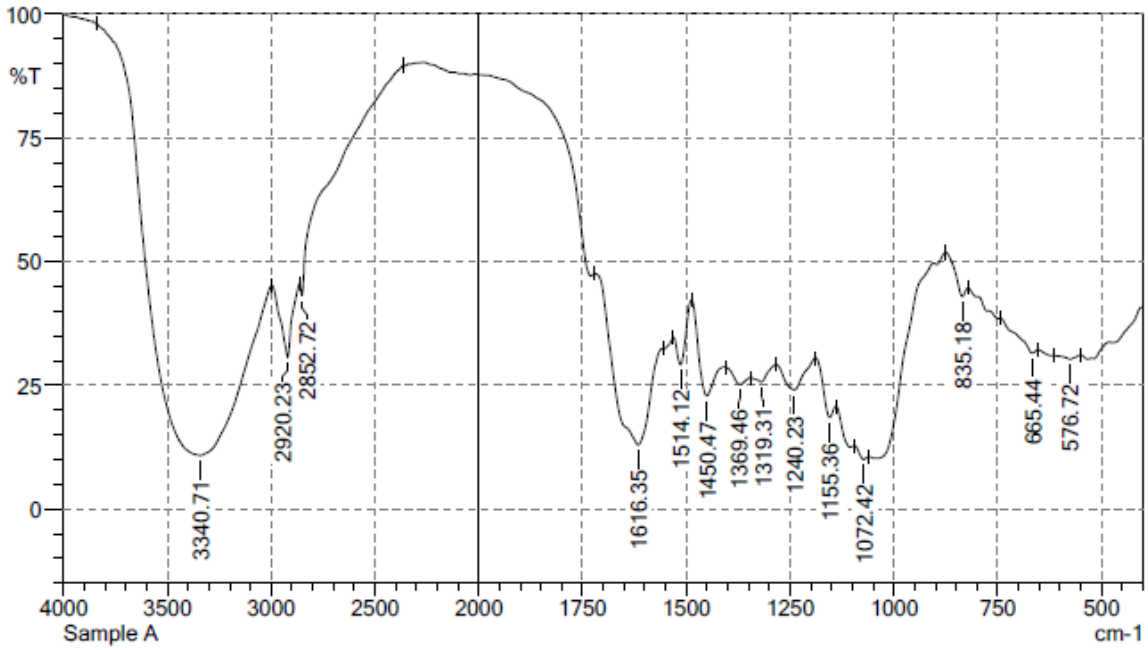
DRUG & POLYMER COMPATABILITY STUDIES BY FTIR:

Pressed pellet technique was used to handle the sample in FTIR spectrometer. In this technique a pinch of sample was mixed with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted in to special holder of IR spectrometer.

IR spectrums for pure drug alone and physical mixture of drug and polymers are taken. The spectrum of physical mixture was compare with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture, hence there was to no significant interaction between drug and excipients

FTIR SPECTRUM

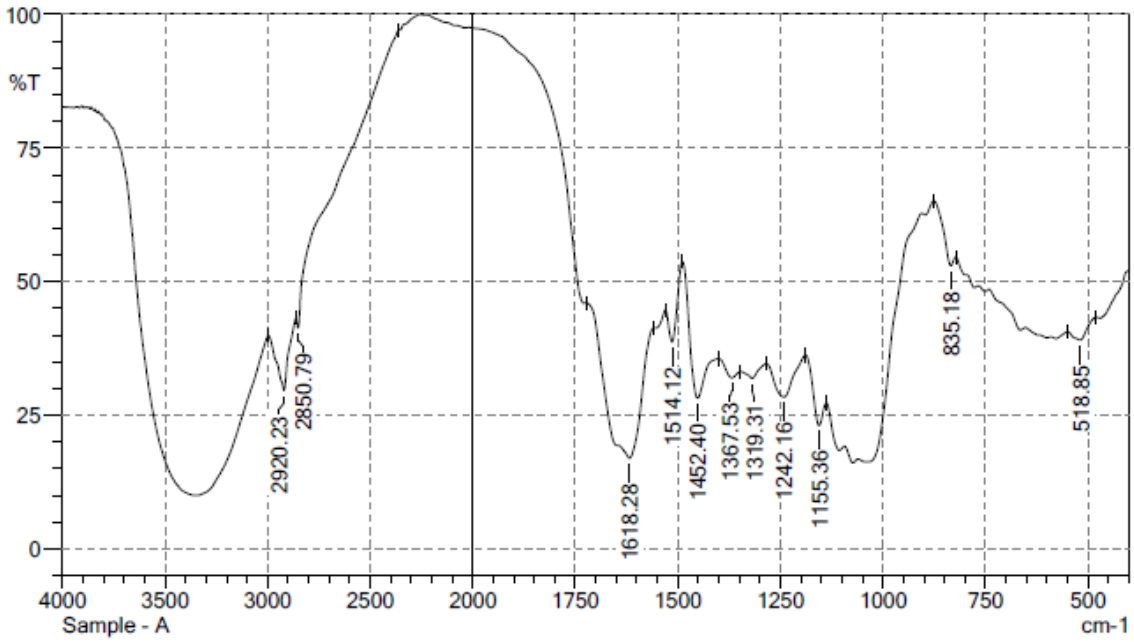
Fig No . 12 FTIR OF *SENNA ALATA LINN*



Functional group	Frequency (cm^{-1})
S=O	835.18
C=C	1354.75
C-C	1514.12
O-H	3340.71
O-H	3726.76

Table No.10 functional group for FTIR Spectra of ethanol leaf extract of *senna alata*

Fig No . 13 FTIR OF *SENNA ALATA LINN NIOSOMES*



Functional group	Frequency (cm^{-1})
S=O	1155.36
C-H	1242.16
C=C	1367.53
C=C	1452.40
C-H	2920.23

Table No.11 functional group for FTIR Spectra of ethanol leaf extract of *senna alata*

6.2.8. *IN VITRO* CHARACTERISATION

Entrapment efficiency of senna alata niosomes

The entrapment efficiency (%) of the niosome formulations (F1, F2, F3, F4, F5, F6, F7) and an optimised formulation F was determined and tabulated.

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

S.No	Formulation code	Amount of the drug Entrapped (mg)	Percentage Entrapment Efficiency
1	F1	36.22±0.54	63.78±0.73
2	F2	31.88±0.24	68.12±0.60
3	F3	25.85±0.36	74.15±0.45
4	F4	51.63±0.17	48.37±0.56
5	F5	30.14±0.12	69.86±0.24
6	F6	1.97±0.19	98.03±0.35
7	F7	21.88±0.32	78.12±1.09

The F6 formulation has showed entrapment efficiency of 98.03±0.35% the formulation F7 showed % entrapment efficiencies of 78.12±1.09%. The decrease in the % entrapment efficiency then compared to formulation F6 was due to increased copolymer concentration because of higher hydrophobic or steric interactions between the polymer and drug.

Hence the F6 formulation has shown highest % entrapment efficiency with higher drug loading content when compared with other formulations. So, it was selected as an optimized formulation.

In vitro drug release profile of senna alata L. Niosomes

The Invitro drug release studies were performed for the prepared Niosome formulations (F1, F2, F3, F4, F5, F6 F7). Results were tabulated. The graph was plotted between cumulative % drug release against time in hours on the y axis and x axis respectively.

The formulation F6 has showed a drug release of 98.76 % in 24 hours. Whereas the F7 showed a drug release of 93.59 % the decrease in drug release when compared with the F6 formulation was found to be due increased hydrophobic or steric interactions between drug and polymer. Hence, the F6 formulation was selected as the best formulation with highest percent drug release and also having the higher % encapsulation efficiency then compared to the other formulation.

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

S.No	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	12.11	12.59
2	2	24.32	24.68
3	3	35.41	35.87
4	4	47.23	47.76
5	5	50.24	50.54
6	6	64.23	64.78
7	7	75.32	75.63
8	8	80.12	80.57
9	9	89.26	89.68
10	10	98.18	98.76

Fig No . 14 In vitro drug release data of F1 formulation

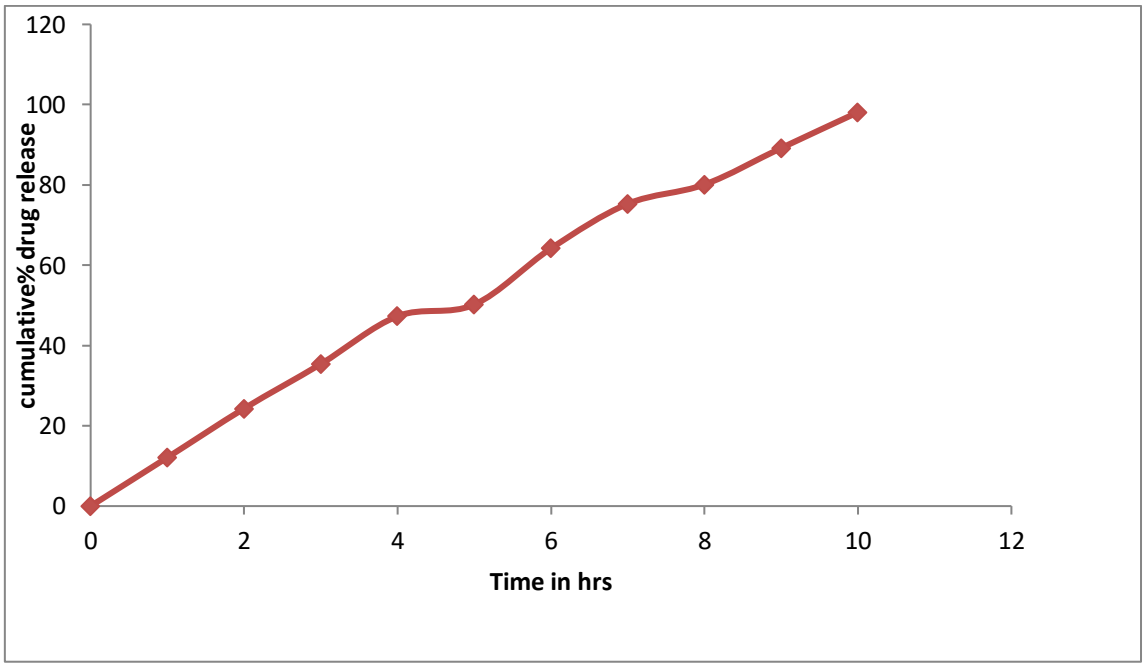


Table No : 14 In vitro drug release data of F2 formulation

S.No	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	4.05	4.25
2	2	7.92	7.98
3	3	11.22	11.54
4	4	15.12	15.36
5	5	21.23	21.43
6	6	26.12	26.35
7	7	32.04	32.15
8	8	37.21	37.43
9	9	42.03	42.23
10	10	48.04	48.15
11	11	55.14	55.23
12	12	65.16	65.46
13	13	71.03	71.34
14	14	74.18	74.25
15	15	77.05	77.35
16	16	80.12	80.20
17	17	84.05	84.16
18	18	87.11	87.22
19	19	89.12	89.23
20	20	90.14	90.20
21	21	93.23	93.28
22	22	95.13	95.35
23	23	98.01	98.54
24	24	99.43	99.65

Fig no : 15 In vitro drug release data of F2 formulation

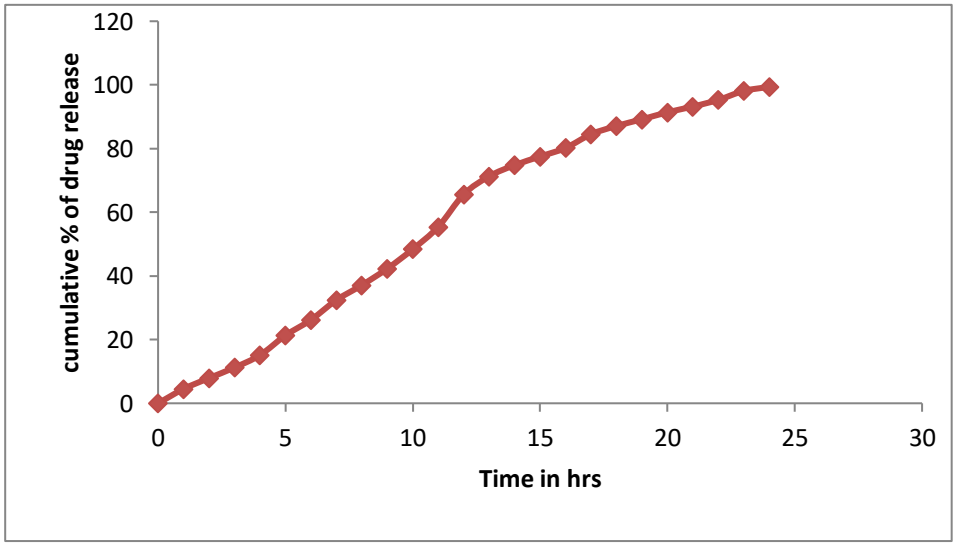


Table No .15 In vitro drug release data of F3 formulation

S.No	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	12.02	12.15
2	2	17.13	17.20
3	3	24.05	24.23
4	4	32.13	32.65
5	5	39.15	39.34
6	6	47.02	47.25
7	7	55.32	55.54
8	8	63.06	63.35
9	9	72.14	72.43
10	10	79.23	79.33
11	11	86.12	86.42
12	12	90.52	90.56
13	13	97.14	97.35
14	14	99.01	99.30

Fig no : 16 In vitro drug release data of F3 formulation

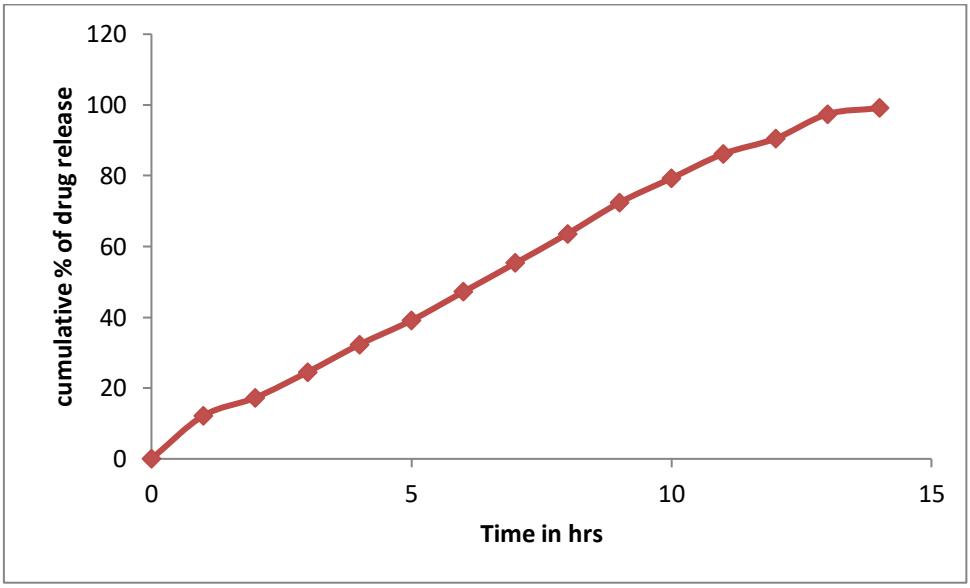


Table No.16 In vitro drug release data of F4 formulation

S.No	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	25.15	25.24
2	2	42.02	42.35
3	3	68.18	68.43
4	4	78.03	78.15
5	5	98.27	98.3

Fig no: 17 In vitro drug release data of F4 formulation

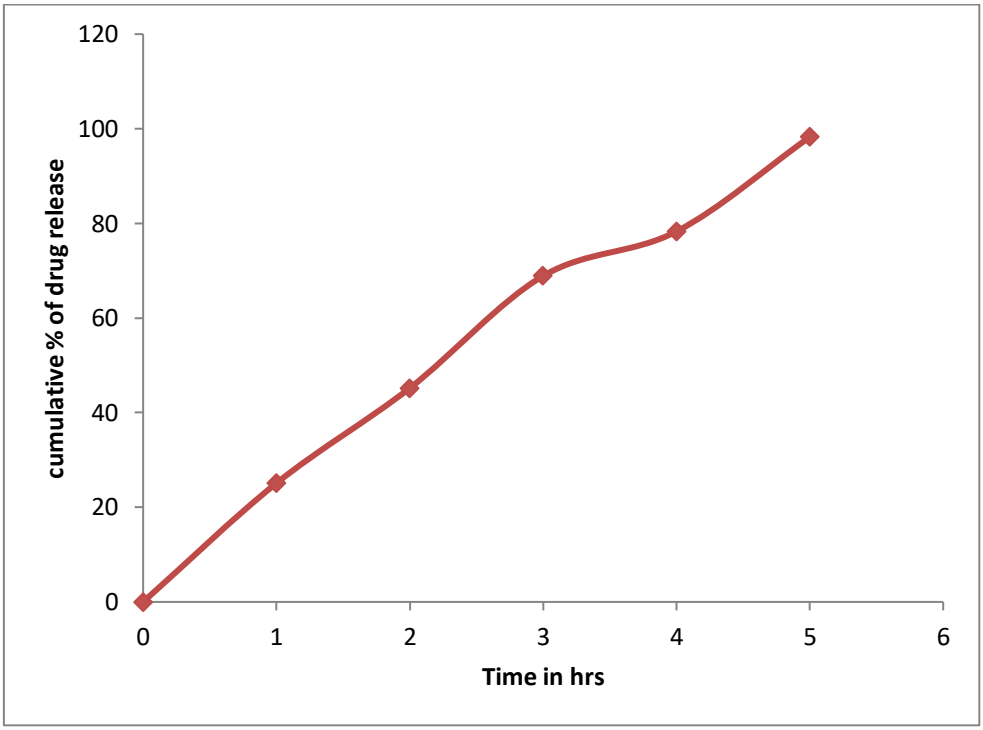


Table No .17 In vitro drug release data of F5 formulation

S.No	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	12.05	12.20
2	2	23.14	23.26
3	3	34.20	34.50
4	4	42.31	42.64
5	5	54.15	54.36
6	6	62.23	62.45
7	7	73.50	73.67
8	8	80.21	80.71
9	9	88.05	88.65
10	10	92.12	92.38
11	11	98.17	98.26

Fig no:18 In vitro drug release data of F6 formulation

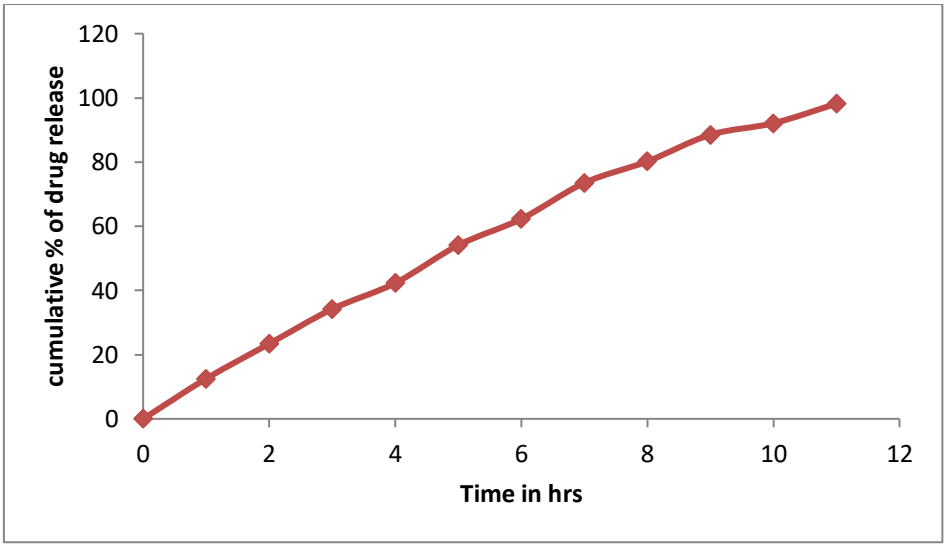


Table No.18 In vitro drug release data of F6 formulation

S.NO	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	5.04	5.12
2	2	8.12	8.23
3	3	12.31	12.42
4	4	16.14	16.23
5	5	19.25	19.31
6	6	23.21	23.27
7	7	26.13	26.24
8	8	30.08	30.36
9	9	34.56.	34.79
10	10	37.05	37.19
11	11	41.25	41.50
12	12	45.13	45.26
13	13	49.15	49.38
14	14	53.27	53.49
15	15	58.16	58.34
16	16	64.27	64.35
17	17	69.15	69.27
18	18	75.08	75.19
19	19	78.22	78.26
20	20	80.15	80.23
21	21	83.12	83.27
22	22	87.25	87.53
23	23	93.21	93.29
24	24	98.45	98.76

Fig. No. 19 In vitro drug release data of F6 formulation

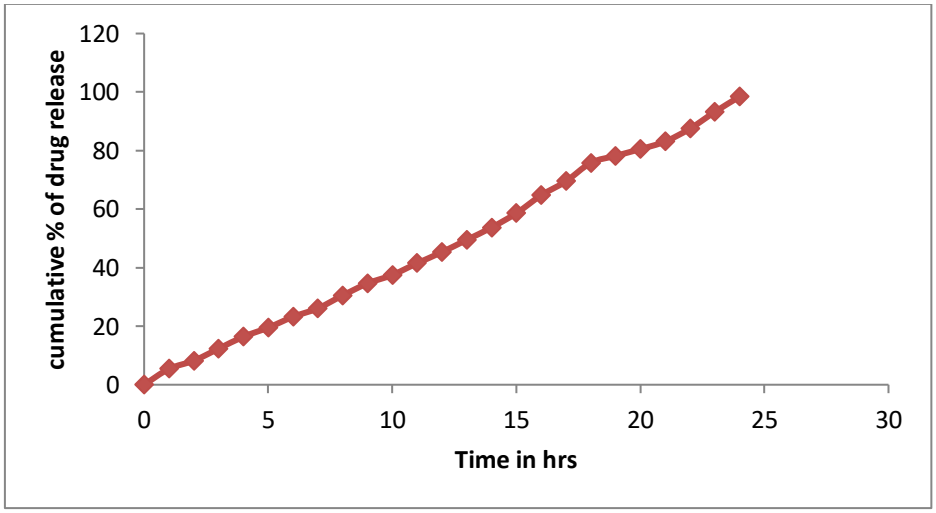
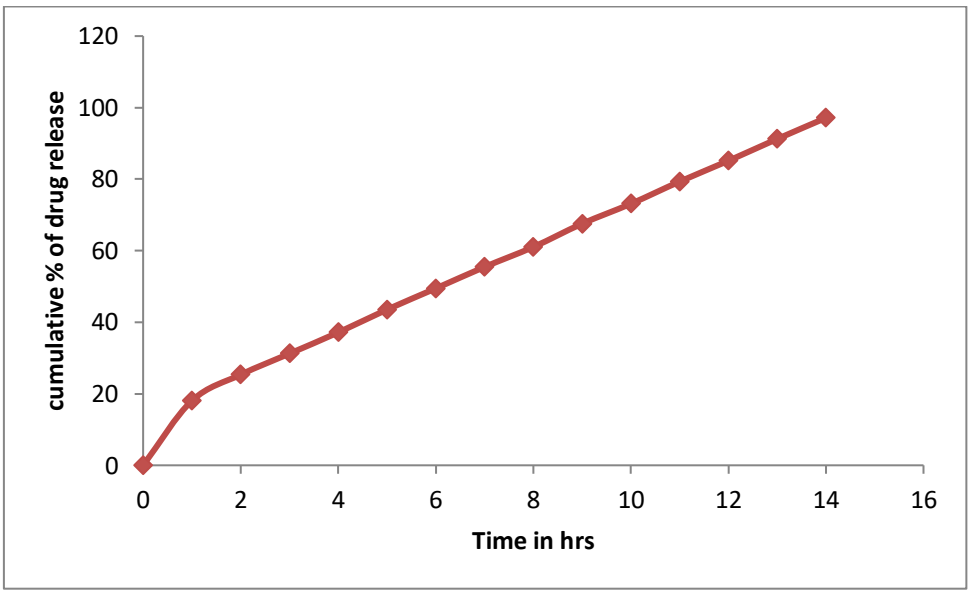


Table No.19 In vitro drug release data of F7 formulation

S.NO	Time (hrs.)	Percentage of drug release	Cumulative % of drug release
1	1	18.05	18.21
2	2	25.34	25.54
3	3	31.23	31.35
4	4	37.14	37.29
5	5	43.53	43.76
6	6	49.45	49.60
7	7	55.25	55.46
8	8	61.04	61.27
9	9	67.54	67.86
10	10	73.12	73.26
11	11	79.32	79.45
12	12	85.14	85.22
13	13	91.24	91.37
14	14	97.12	93.59

Fig no: 20 In vitro drug release data of F7 formulation



6.2.9. Stability Study

The optimized formulation F6 was subjected to stability study for one month at 4^0 C (refrigerator), room temperature and at 45^0C/75% RH. At the interval of 30 days, samples of niosomal formulation were taken and evaluated for the entrapment efficiency and in vitro release of drug Entrapment efficiency of optimized formulation F6 kept at 4^0C 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 at room temperature and at 45^0C/75%RH were 92% and 81% respectively after 30 days of stability study

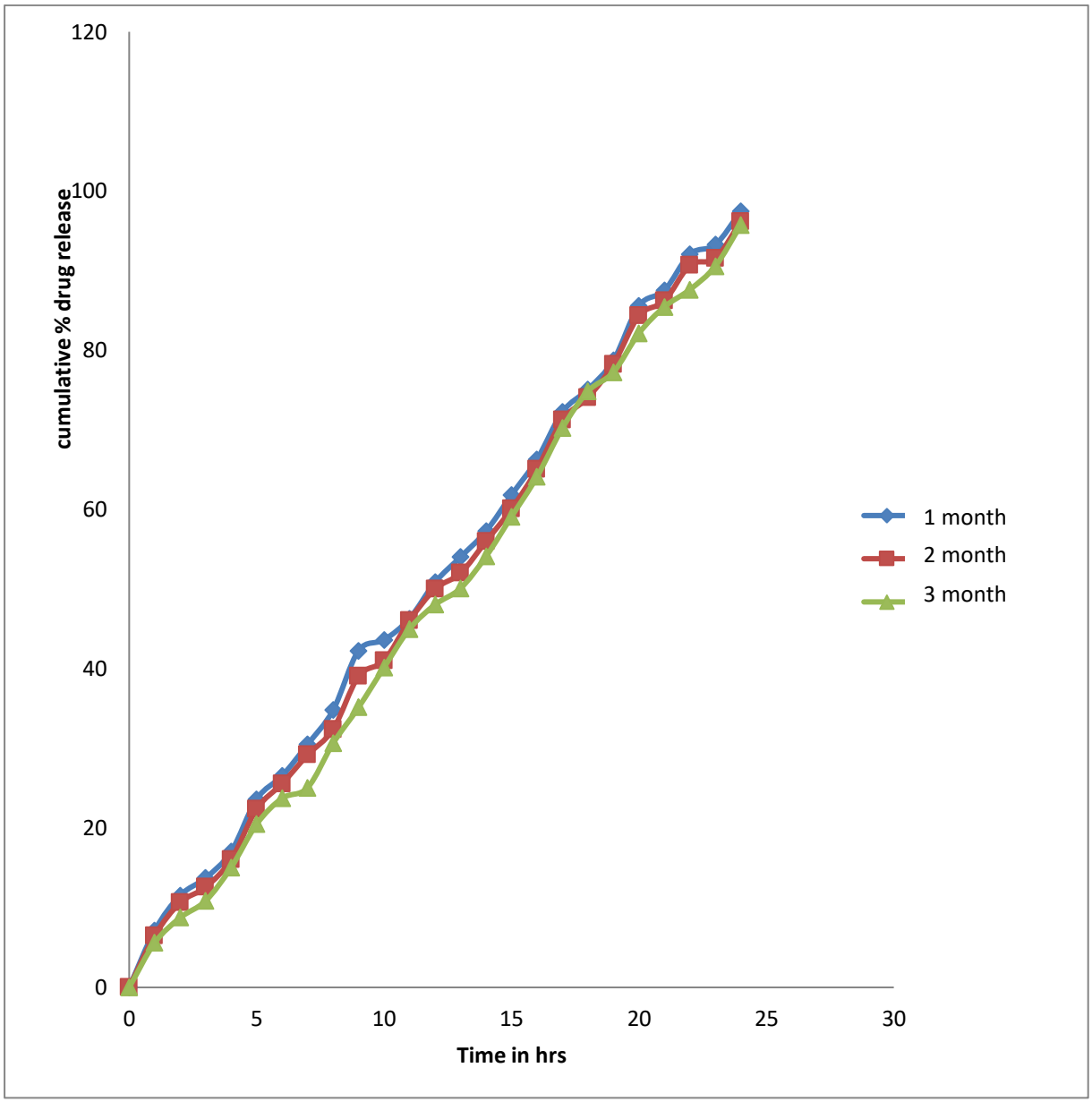
The in vitro release of optimize formulation F6 shows that the niosomal formulation are more stable at 4^0 C (Refrigerator) when compared to room temperature and at 45^0C/75%RH (stability chamber). The niosomal formulation kept at 4^0 C showed a cumulative release of 89.89% after 30 days of stability studies.

STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT 4^0C

Table No : 20

Time in hours	Cumulative % Drug release		
	After 1 Month	After 2 Month	After 3 Month
1	7.14	6.5	5.6
2	11.55	10.65	8.75
3	13.75	12.59	10.87
4	17.05	16.05	15.06
5	23.63	22.04	20.05
6	26.57	25.59	23.75
7	30.52	29.02	25.08
8	34.81	32.41	30.65
9	42.29	39.07	35.17
10	43.64	41.05	40.18
11	46.32	46.08	45.02
12	50.86	50.02	48.06
13	54.05	52.03	50.11
14	57.28	56.05	54.16
15	61.84	60.08	59.12
16	66.29	65.05	64.15
17	72.26	71.25	70.26
18	75.09	74.08	74.86
19	78.74	78.26	77.25
20	85.58	84.35	82.15
21	87.53	86.21	85.46
22	92.08	90.69	87.63
23	93.28	91.55	90.58
24	97.46	96.16	95.75

Fig No. 21 STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT 4^0C



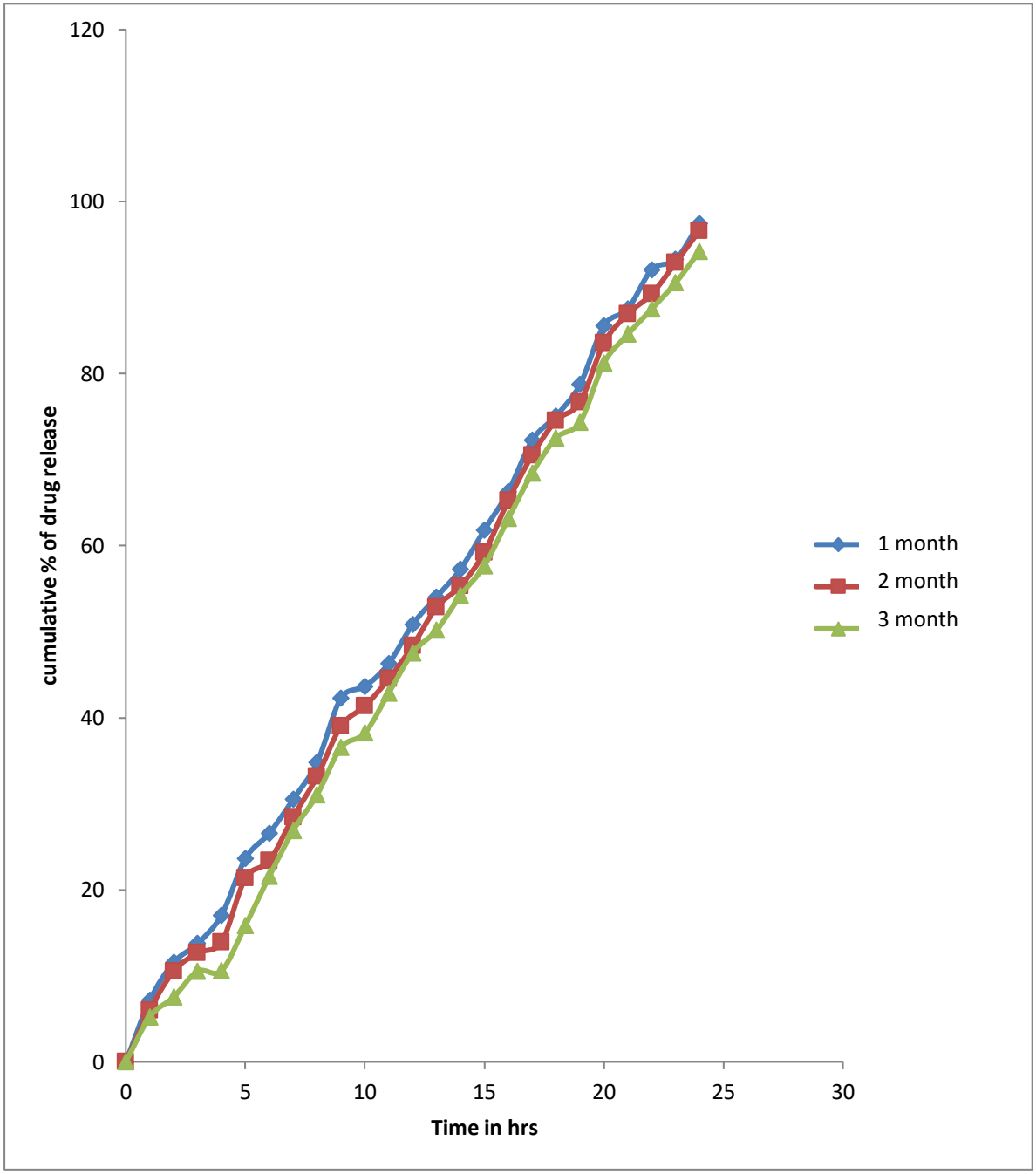
`

Table No. 21 STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT ROOM TEMPERATURE

Time in hours	Cumulative % Drug release		
	After 1 Month	After 2 Month	After 3 Month
1	7.14	6.01	5.23
2	11.55	10.53	7.53
3	13.75	12.68	10.54
4	17.05	13.89	11.58
5	23.63	21.35	15.85
6	26.57	23.36	21.56
7	30.52	28.45	26.89
8	34.81	33.16	31.05
9	42.29	39.02	36.53
10	43.64	41.36	38.23
11	46.32	44.53	42.89
12	50.86	48.36	47.56
13	54.05	52.85	50.18
14	57.28	55.32	54.23
15	61.84	59.23	57.68
16	66.29	65.23	63.15
17	72.26	70.53	68.43
18	75.09	74.56	72.53
19	78.74	76.65	74.35
20	85.58	83.56	81.23
21	87.53	86.95	84.57
22	92.08	89.26	87.52
23	93.28	92.89	90.57
24	97.46	96.64	94.23

STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT ROOM TEMPERATURE

Fig no: 22



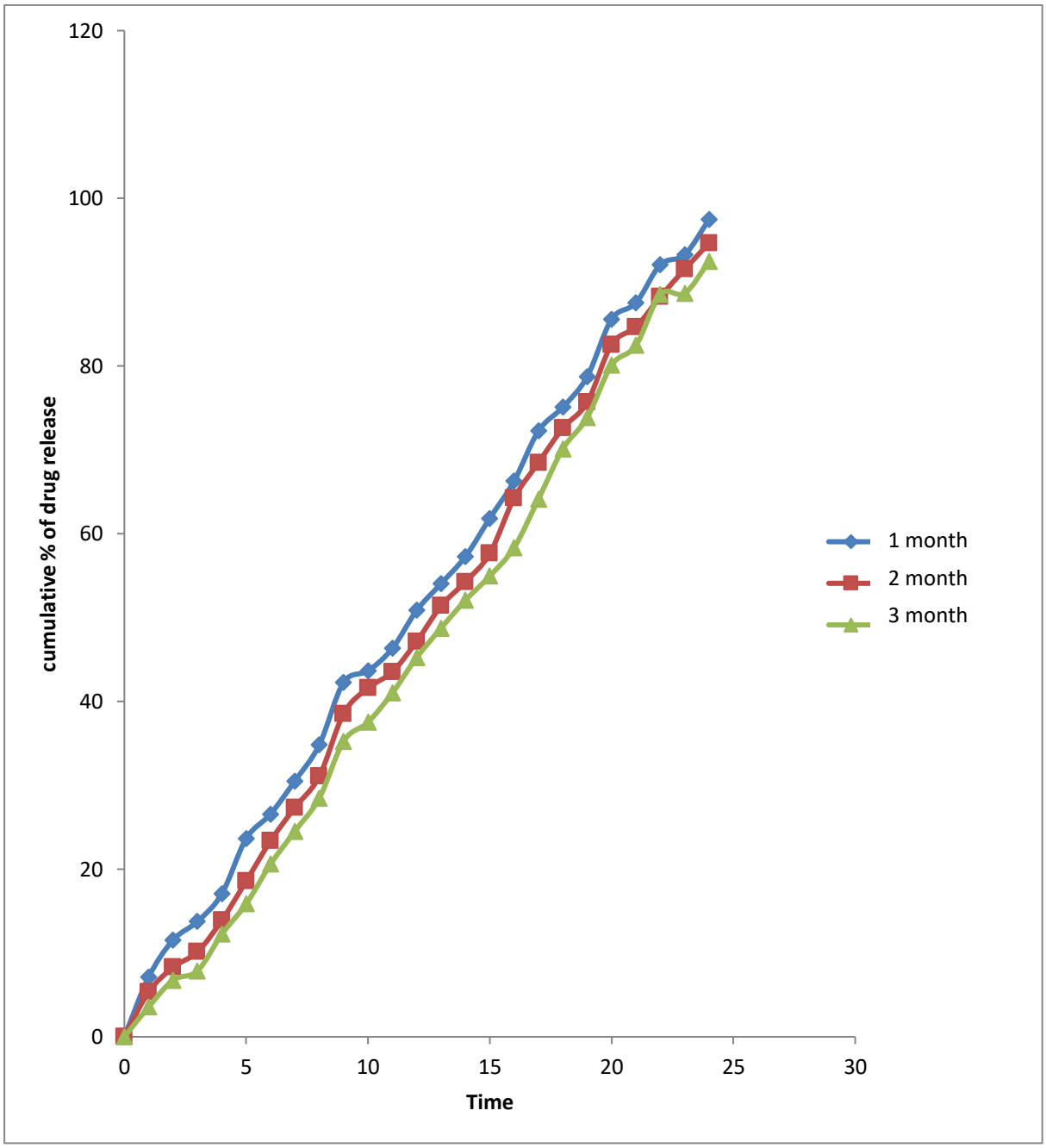
STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT 45ºC

Table No : 22

Time in hours	Cumulative % Drug release		
	After 1 Month	After 2 Month	After 3 Month
1	7.14	5.36	3.58
2	11.55	8.29	6.76
3	13.75	10.13	7.86
4	17.05	13.89	12.25
5	23.65	18.57	15.85
6	26.57	23.36	20.59
7	30.52	27.35	24.53
8	34.81	31.05	28.45
9	42.29	38.54	35.26
10	43.64	41.59	37.52
11	46.32	43.52	41.02
12	50.86	47.16	45.23
13	54.05	51.39	45.23
14	57.28	54.26	48.75
15	61.84	57.64	52.06
16	66.29	64.25	54.96
17	72.26	68.49	58.35
18	75.09	72.59	64.15
19	78.74	75.67	70.12
20	85.58	82.56	73.86
21	87.53	84.57	80.11
22	92.08	88.25	82.45
23	93.28	91.54	88.56
24	97.46	94.65	92.45

STABILITY STUDY RELEASE DATA FOR FORMULATION F6 AT 45^{0}C

Fig No : 23

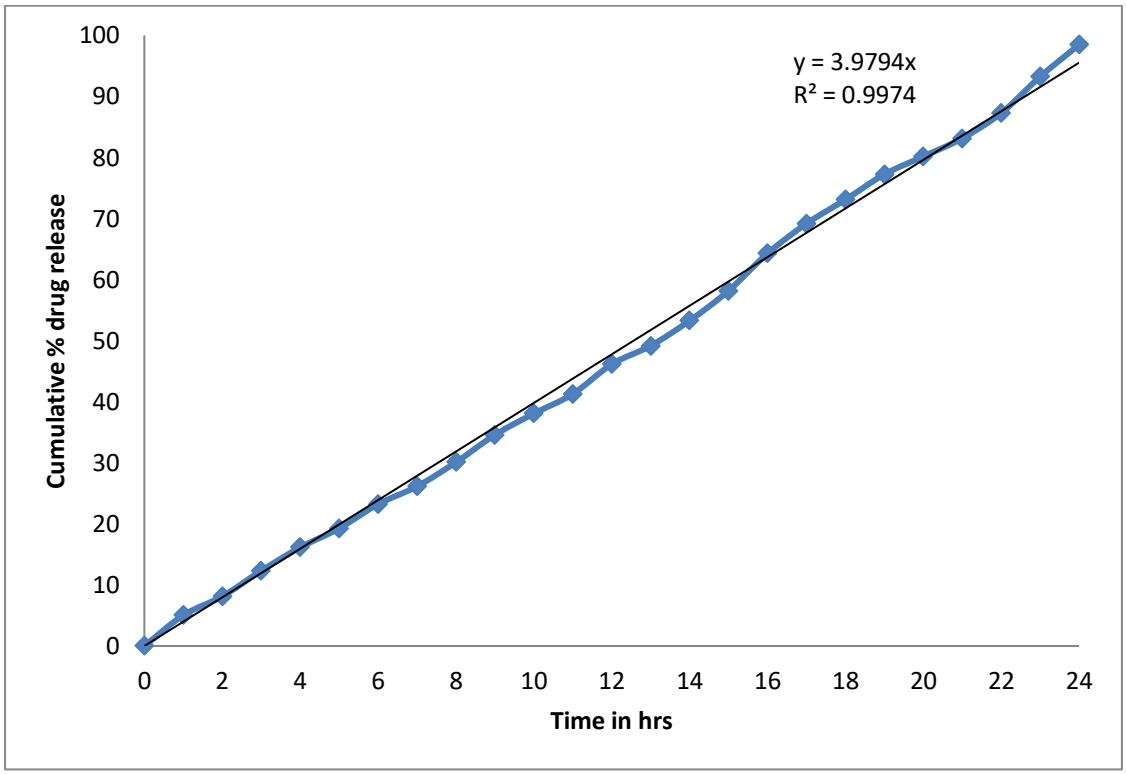


6.3. RELEASE KINETICS STUDIES

The release kinetics was studied for an optimised formulation F6 by plotting the graphs for different kinetic models by using the in vitro release data

Zero Order Plot:

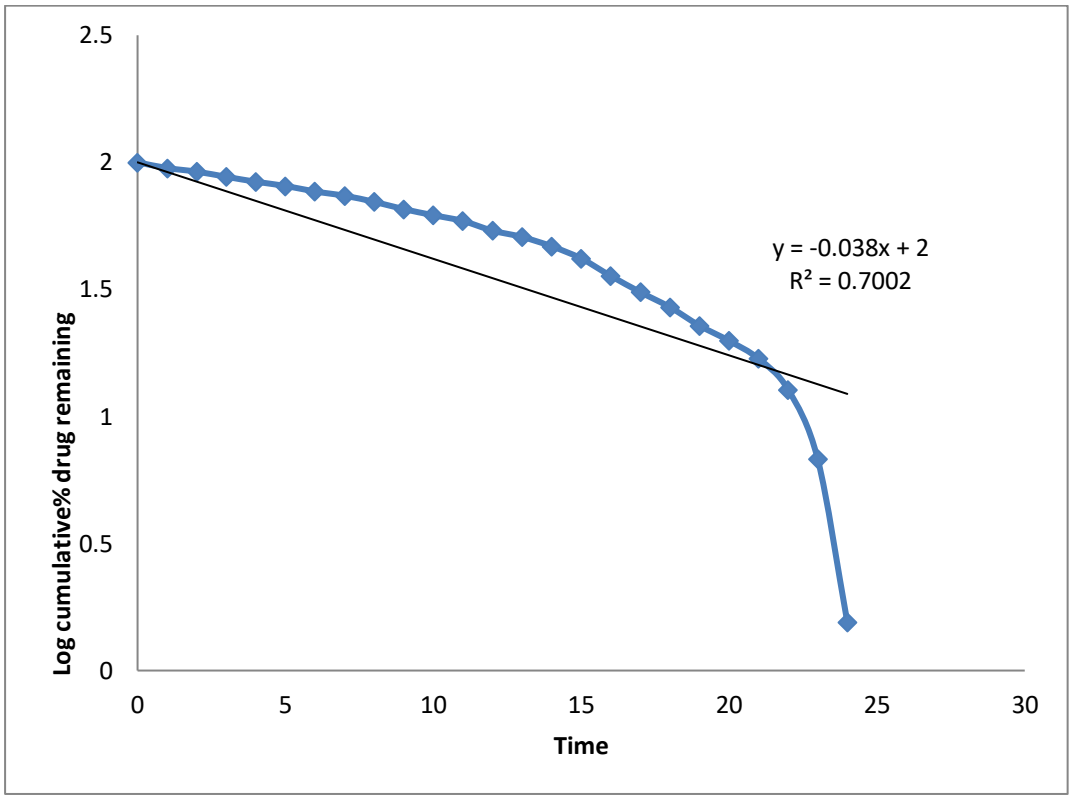
Fig No: 24 Zero Order release profile for an optimized F6 formulation



Zero Order release kinetics	
R^2	0.997

First Order Plot:

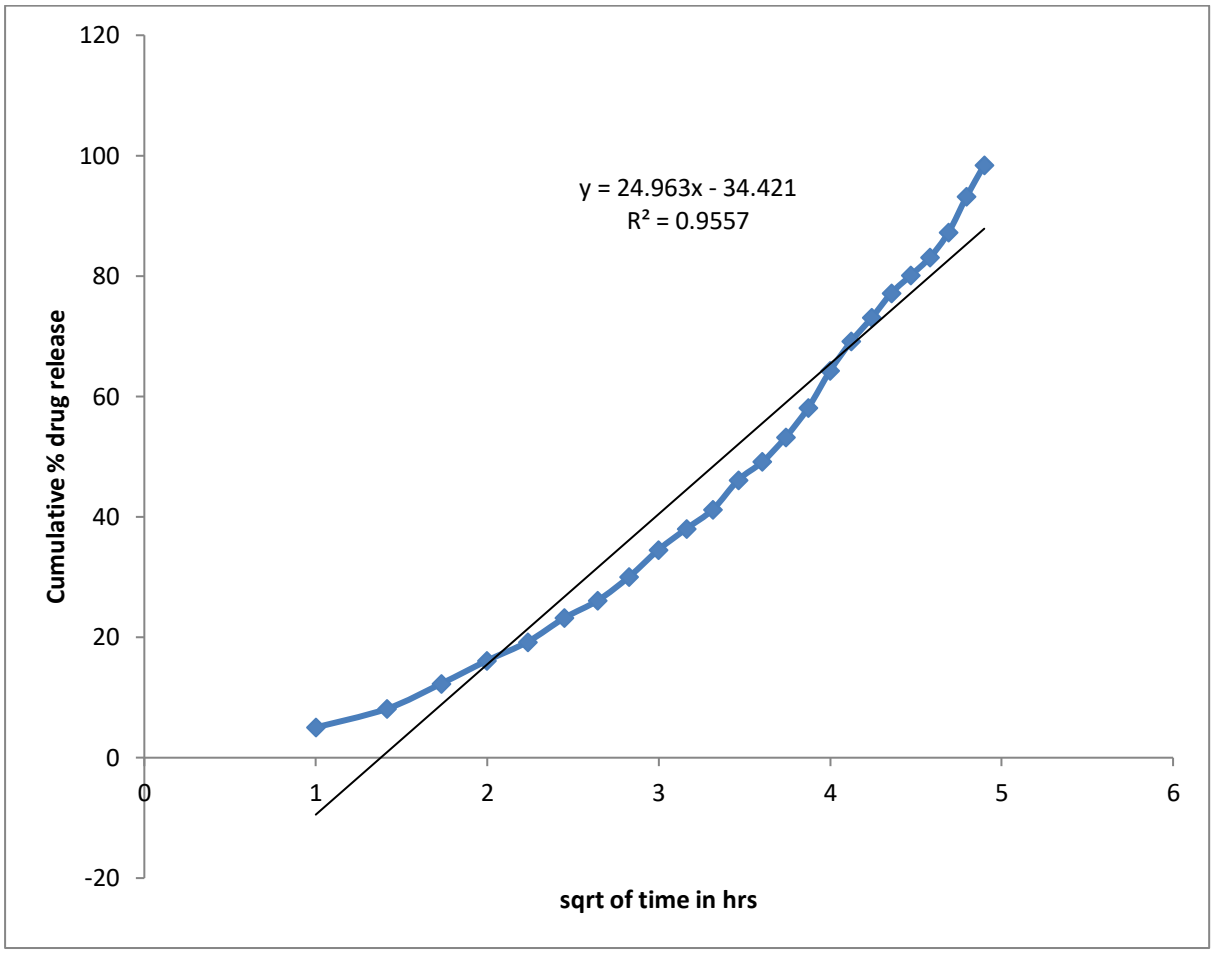
Fig. No: 25 First Order release profile for an optimized F6 formulation



First Order release kinetics	
R^2	0.700

Higuchi Plot:

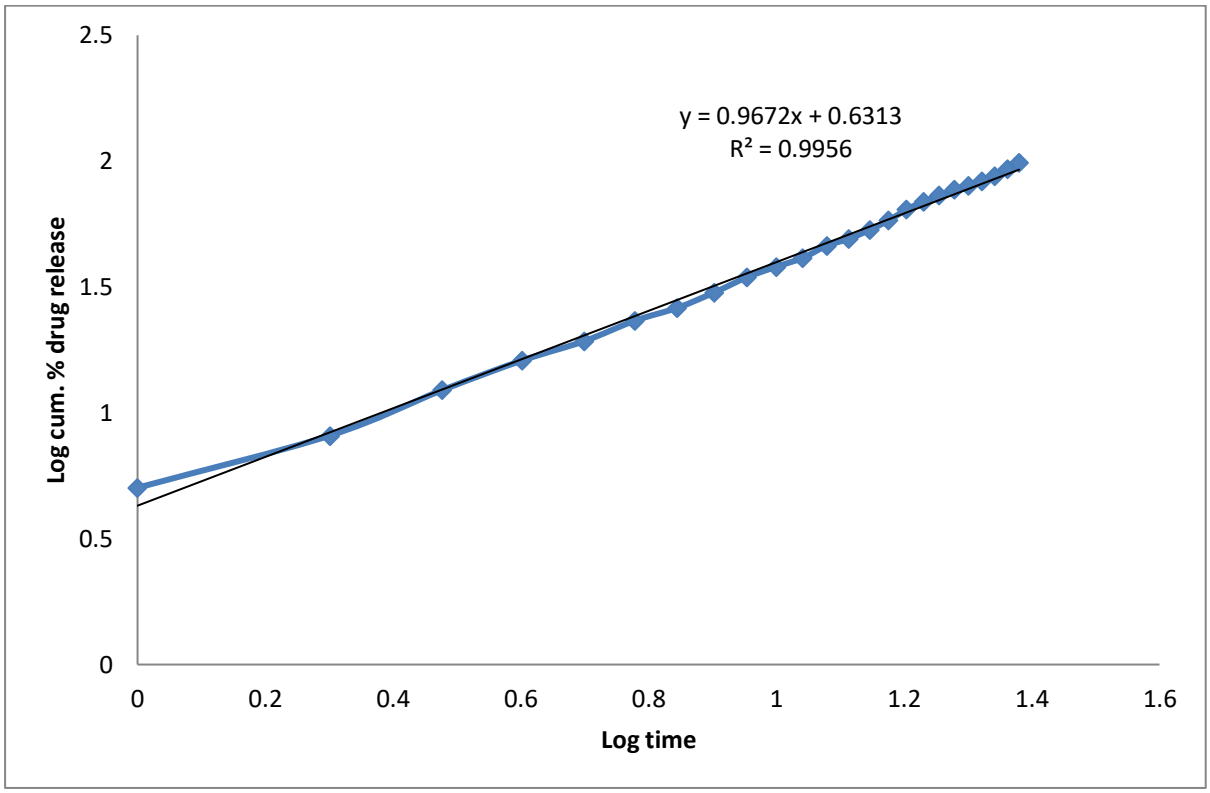
Fig No: 26 Higuchi release profile for an optimized F6 formulation



Higuchi release kinetics	
R^2	0.956

Korsmeyer-Peppa's model kinetics Plot:

Fig No: 27 Korsmeyer-Peppa's release profile for an optimized F6 formulation



Korsmeyer-Peppa's model release kinetics	
n value	**0.631**

Zero Order plot:

Zero order plot of a freeze dried formulation F4 was to be linear with a regression value of 0.997, which signifies that the drug was released in a controlled manner from the nanoparticles during the release study.

First Order plot:

First Order plot was made by plotting log remaining cumulative % drug release against time and the regression value was found to be 0.700 which indicates that drug release was not followed the first order rate kinetics.

Higuchi plot:

Higuchi plot was found to be linear with a regression value of 0.956, which indicates that diffusion was one of the mechanisms of the drug release from nanoparticles matrices.

Korsmeyer-Peppa's Plot:

The type of in vitro mechanisms of drug release was best explained by Korsmeyer – Peppa's plot. The plot was found to be linear with R^2 value 0.996 and diffusion exponent 'n' value was 0.631 According to Korsmeyer-Peppa's equation, mechanisms of drug based on 'n' which indicates that mechanism of drug release from copolymer matrices was followed non Fickian diffusion.

So, the kinetic studies of an optimized F6 formulation with different kinetic models has showed that the senna alata L. release profile was best fitted with zero order plot and release mechanism was found to be non Fickian diffusion based on 'n' value from Korsmeyer-Peppa's plot.

6.4. Morphology

6.4.1. Binocular microscopy

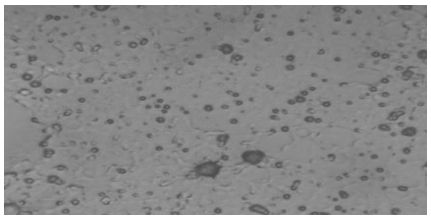


Fig No 28 Binocular microscopy

Scanning Electron Microscopy (SEM)

The niosomes morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscopy (SEM) at different magnifications. A few mg of prepared niosomes was gold coated using a Hitachi HVSJGB Vacuum evaporator. Coated samples were viewed and photographed in a Hitachi S-450 SEM operated at 20 k v

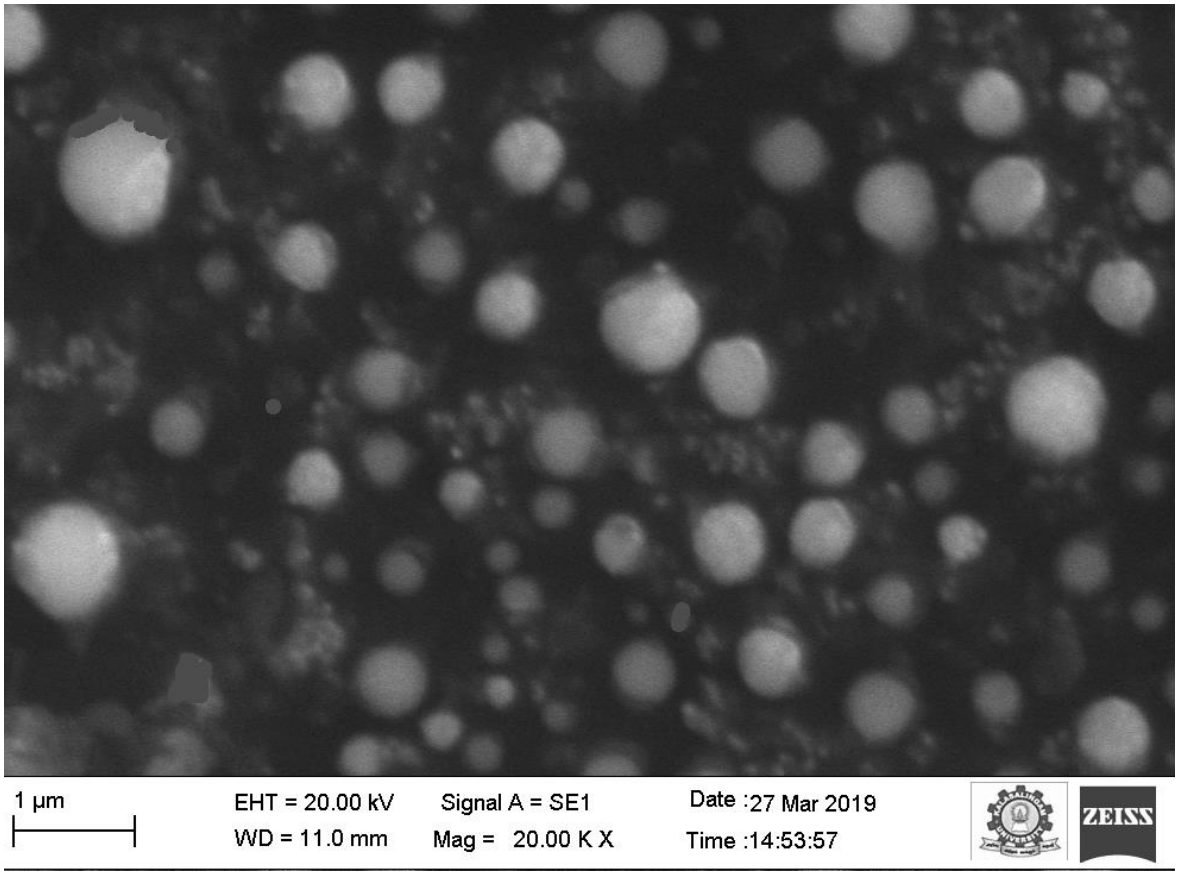


Fig No: 29 SEM Photograph

6.5. Evaluation of Antimicrobial Activity of Senna Alata L.niosomal gel formulation

The Antimicrobial Activity of Senna Alata L.niosomal gel formulation was carried out by cup plate method

Media composition:

Table No.23 MGYP medium: (pH: 6.4-6.8)

S.No	Ingredient	Quantity taken
1.	Malt extract	0.6 g
2.	Glucose	2.0 g
3.	Yeast extract	0.6 g
4.	Peptone	1.0 g
5.	Distilled water	200.0 ml
6.	Agar	4.0 g

Procedure of media preparation:

Fig No. 30 Sterilization of MGYP medium, Nutrient Agar medium, Potato Dextrose Agar medium by Autoclaving

MGYP medium:

- The respective quantity of Malt extract, Glucose, Yeast extract, Peptone, Agar were added in 200ml of distilled water.
- Heat to boiling to dissolve the medium completely
- Then sterilized by autoclaving 15 lb at 121^0C for 15 min.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification. 15µl of inoculums of the bacteria was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 37^0C $\pm1^0$C for 24 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Table No.24 Nutrient Agar medium: (pH: 7-7.5)

S.No	Ingredient	Quantity taken
1.	Beef extract	2.0 g
2.	NaCl	1.0 g
3.	Peptone	2.0 g
4.	Distilled water	200.0 ml
5.	Agar	4.0 g

Nutrient Agar medium:

- The respective quantity of Beef extract, NaCl, Peptone, Agar adds in 200ml of distilled water.
- Heat to boiling to dissolve the medium completely
- Then sterilized by autoclaving 15 lb at 121^0C for 15 min.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification. 15µl of inoculums of the bacteria was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 37^0C ±1^0C for 24 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Table No.25 Potato Dextrose Agar medium: (pH: 6-7)

S.No	Ingredient	Quantity taken
1.	Pieces of potato	40.0 g
2.	Dextrose	4.0 g
3.	Yeast extract	0.02 g
4.	Distilled water	200.0 ml
5.	Agar	4.0 g

Potato Dextrose Agar medium:

- 40.0 g of peeled potatoes are cut into small pieces and suspended in 200.0 ml of distilled water.

- Steamed for 30 min. decant the extract or filter through muslin cloth and make the final volume to 200.0 ml.
- Add 4.0 g of dextrose, 0.02 g of yeast extract and 4.0 g of agar.

The respective medium was sterilized by autoclaving at 121^0C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow solidification..15µl of inoculums of the bacteria and fungi was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 28^0C for 48 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Preparation of inoculums:

Fungal inoculum:

The inoculums was prepared by inoculate the Spores of in Potato dextrose broth and *Candida albicans,* Aspergillus niger MGYP broth and incubated at 37^0C for 24 hrs.

Bacterial inoculums:

The inoculums was prepared by inoculate the Spores of *Escherichia coli, pseudomonas aureus, Staphylococcus aureus in* Nutrient broth and incubated at 37^0C for 24 hrs.

Preparation of sample:

Stock solution: 100mg of drug is dissolved in 10ml of methanol to produce 10000µg concentration.

Sample: From this stock solution different dilution were prepared in the concentration rang of 1,2,3,4,5,6,7,8,9,10 mg/ml in 10ml of volumetric flask.

Agar cup plate method:

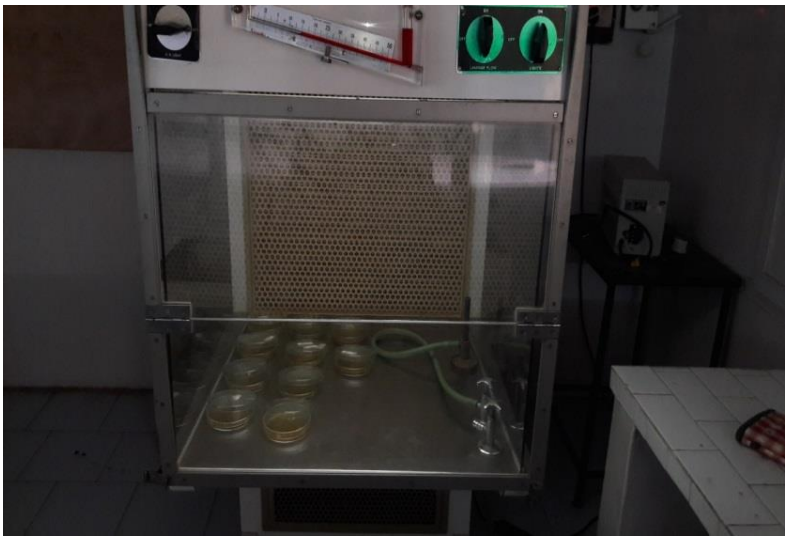


Fig No: 31 Laminar air flow

- The respective medium was sterilized by autoclaving at 121^{0}C (15lb/ln^2).for 15 min. and medium was transferred aseptically into sterilized glass Petri plates.
- The plates were left at room temperature to allow solidification.15µl of inoculums of the bacteria and fungi was transferred to respective Petri plate. Four wells of 6mm diameter were made using a sterile borer.
- The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were kept at straight place for 2 hours to allow the diffusion of the solution into the medium.
- The Petri dishes was incubated in an inverted position. For antibacterial screening were incubated at 37±1 ^{0}C for 24 hours, while those used for antifungal activity were 28^{0}C for 48 hours. The diameter of zone of inhibition was recorded. And tabulated the result.

Determination of Zone of Inhibition of Senna alata niosomal gel by agar Cup Plate method:

Fungal and bacterial strain:

The bacterial and fungal strains employed in the study were obtained from the National collection of industrial microorganisms (NCIM) Pune.

Table No.26 Fungal strain:

S. No.	Name of organism	Maintenance Media
1.	*Aspergillus niger*	Potato Dextrose Agar
2.	*Candida albicans*	MGYP
3.	*Candida albicans*	MGYP

Table No.27 Bacterial strain:

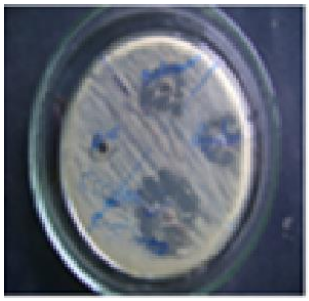
S. No.	Name of organism	Maintenance Media
1.	*Pseudomonas aeruginosa*	Nutrient Agar
2.	*Staphylococcus aureus*	Nutrient Agar
3.	*Escherichia coli*	Nutrient Agar

Antifungal and Antibacterial Evaluation

Table No. 28 Antifungal activity

Name of the fungal pathogens	Senna Alata L. Diameter of zone of inhibition (mm) Concentration (mg/mL)			
	25mg (mg/mL)	50 (mg/mL)	75 (mg/mL)	100 (mg/mL)
Aspergillus niger	16	22	23	25
Candida albicans	8	12	14	17
Candida albicans	14	16	18	21
Fluconazole (500 µg/ml)	18	22	23	26

Aspergillus niger



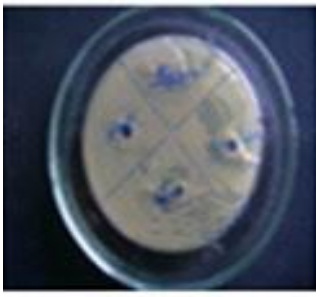
Candida albicans

Candida albicans

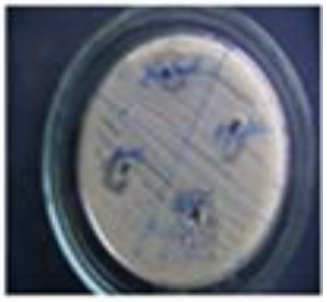
Fig No: 32 Antifungal activity Determination of Zone of Inhibition of Senna alata niosomal gel by agar Cup Plate method:

Table No. 29 Antibacterial activity

Name of the Bacterial pathogens	Senna Alata L. Diameter of zone of inhibition (mm) Concentration (mg/mL)			
	25mg (mg/mL)	50 (mg/mL)	75 (mg/mL)	100 (mg/mL)
Escherichia coli	8	11	13	15
Staphylococcus aureus	9.55	12	16	19
Pseudomonas aeruginosa	9	12	14	16
Ciprofloxacin 50µg	20	25	28	32



Escherichia coli



Staphylococcus aureus

Pseudomonas aeruginosa

Fig No: 33 Antibacterial activity Determination of Zone of Inhibition of Senna alata niosomal gel by agar Cup Plate method:

Discussion:

Traditionally medicinal plants have been used for many years as topical and internal preparation in the treatment of fungal and bacterial disease. Many investigations are being carried out throughout the world to discover plant products to inhibit the clinically important fungal pathogens.

The World Health Organization (WHO) estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Each and every country has their own indigenous system of medicine and many of the formulations were not validated for their purported claims and it is the need of the hour to scientifically prove the claimed effects to spread those systems to gain acceptance at the global level.

This study shows that Senna alata has antibacterial & Antifungal activity against Staphylococcus aureus, and has high potential as antibacterial agent when formulated as for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions.

The potency of the Senna alata niosome gel against the bacterial and fungal commonest etiologic agent of boils, carbuncles, infantile impetigo and wounds.

The senna alata niosome gel is better penetrating to the skin & soft smoothening, active against bacterial and fungal

The results also revealed that the extracts incorporated into the gel bases showed better activity than that of the crude extract of Senna alata.

This implied that there might have been better diffusion of drug for the niosome gel than for the crude extract.

Skin disease commonly found on the hands, face and in deep layers of the skin

7. CONCLUSION

An effort was made to formulate the Senna alata Niosomes and incorporate the Niosomes into the gel.

From the results of the present experiments it may be concluded that formulation F6 showing high percentage of entrapment and desired sustained release of Senna alata Niosome gel. Hence F6 formulation was optimized one.

The optimized formulation F6 was found to follow zero order release pattern which was revealed by the linearity shown from the plot of time versus concentration.

Niosomes were stable for 4 weeks at 4^0 C and affirm the drug leakage increased the higher temperature. Optimized formulation was released from the Niosomal gel formulation in a sustained manner for 24 Hours.

The present work revels that the *senna alata* niosomal gel produces anticipated synergistic effect against most of the tested fungal strain.

So, my work revels that the formulated Antimicrobial niosomal gel will shows the benefit to society against opportunistic fungal and bacterial infection.

Antimicrobial activity indicates that the gel preparation of the optimized formulation F6 Thus, the aim of the project was achieved by optimizing the formulation Parameters.

Antimicrobial sensitivity screening, the ethanolic extract of *Senna alata* niosomal gel showed excellent activity and Most of these organisms are natural flora of the skin.

Hence, plant based medicines that inhibit their growth without harming the host represent potential therapeutic agent.

BIBLIOGRAPHY

1. Aarti jag tap, Formulation and Evaluation of niosome entrapped pentoxifylline using *in vivo* bronchodilator activity in guniea pigs ; *India journal of pharmaceutics* 2001;63(1);49-54.
2. Aarti jag tap, Study of antiparkinsonisms activity of plain and niosomal pentoxifylline; *India journal of pharmaceutics* 2001; 63 (3); 239-243.
3. Abbas paradakht, *Invitro* study of polyethylene alkyl ether niosomes for delivery of insulin; *International journal of pharmaceutics* 2007; 328; 130-141.
4. Bertrand, N.; Wu, J.; Xu, X.; Kamaly, N.; Farokhzad, O.C., Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology*. Advanced drug delivery reviews* 2014; 66: 2-25.
5. Ajith Singh design and development of niosomal delivery system; *Pharma time*, 2004; 36: 11-13.
6. Anna m.fadda niosomes carries for Tretinoin III. Study into the in vitro cutaneous delivery of vesicle incorporated Tretinoin; *International journal of pharmaceutics*, 2006; 311; 11-19.
7. Biju S.S. vesicular system; an over view *Indian journal of pharmaceutical science*, 2006; 68(2); 141-153.
8. Hu C., Rhodes D.G., Proniosomes: a novel drug carrier preparation. *Int. J. Pharm.* 1999; 185: 23-35.
9. Yoshioka T., Stemberg B.,Florence A.T., Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60, and 80) and a sorbitan triester (Span 85). *Int. J Pharm*.1994; 105: 1-6.
10. Yoshida H., Lehr, C.M., Kok W., Junginger H.E., Verhoef J.C., Bouwistra J.A,. Niosomes for oral delivery of peptide drugs*. J.Control Rel*.1992; 21: 145-153.
11. http//en.wikipedia.org/wiki/Niosomes, structure of niosomes. -
12. Roberson A., Cummings J., Wilmot N., Florence AT., The distribution of doxorubicin in mice following administration in niosomes. *J. Pharm. Pharmacol.* 1988; 40: 337-342.
13. Satturwar, P.M., Fulzele, S.V., Nande, V.S., Khandare, J.N., Formulation and evaluation of ketoconazole Niosomes. *Indian J.Pharm*, 2002; 64 (2): 155-158.
14. Vyas S.P., Khar R, K., Niosomes *Targeted and Controlled Drug delivery*, 2008; 249 – 279.

15. Gibaldi .M and Perrier D; Pharmacokinetics, second edition, *New York, Marcel Dekker, Inc*., 1982; 127-134.
16. Namdeo, A., Jain, N.K., Niosomal delivery of 5-fluorouracil. *J. Microencapsulate*. 1999; 16 (6): 731 – 740.
17. Bhaskaran S., Panigrahi L., Formulation and Evaluation of Niosomes using Different Non-ionic Surfactant. *Ind J Pharm Sci.* 2002; 63: 1-6.
18. Balasubramaniam A., Formulation and In-Vivo Evaluation of Niosome Encapsulated Daunorubicin Hydrochloride. *Drug Dev and Ind.Pharm*.2002; 3(2): 1181-84. 21
19. Schreiber H., Liposomes and niosomes as topical drug carriers: *dermal and transdermal delivery. J. Controlled Release*. 1985; 30: 863-868.
20. Buck ton G., Harwood, Interfacial phenomena in Drug Delivery and Targeting *Academic Publishers, Switzerland*. 1995; 154-155.
21. Hunter C.A., Dolan T.F., Coombs G.H., Baillie A.J, Vesicular systems (niosomes and liposomes) for delivery of sodium stribogluconate in experimental murine visceral Leishmaniasis. M.pharm. *Pharmacol.* 1988; 40 (3): 161-165.
22. Bairwa N. K., Choudhary Deepika., Pro niosome: A review, *Asian Journal of Biochemical and Pharmaceutical Research*. 2011; 2 (1): 690-694.
23. Suzuki K., Sokan K., The Application of Liposome's to Cosmetics. *Cosmetic and Toiletries.* 1990; 105: 65-78.
24. Chauhan S and Laurence MJ. The Preparation of Polyoxyethylene Containing Non-Ionic Surfactant Vesicles. *J Pharm. Pharmacol*.1989; 41:6.
25. Yoshioka T, Sternberg B and Florence AT. Preparation and Properties of Vesicles (Niosomes) of Sorbitan Monoesters (Span 20, 40, 60, and 80) and a Sorbitan Triester (Span 85). *Int J Pharm.* 1994; 105:1-6.
26. Gayatri DS, Venkatesh P and Udupa N. Niosomal Sumatriptan Succinate for Nasal Administration. *Int J Pharm Sci*. 2000; 62 (6):479-481.
27. Hu C and Rhodes D.G. Proniosomes: a novel drug carrier preparation. *Int J Pharm*.1999; 185:23-35.
28. B.L. Silver Ed., the Physical Chemistry of Membranes, Alan & Unwin and Soloman Press*. New York, USA.*1985; 209-230.

29. Ozer AY. A Novel Drug Delivery System Non -ionic Surfactant Vesicles. *Euro J Pharm Bio pharm.*1991; 75.
30. Uchegbu FI and Vyas PS. Non-Ionic Surfactant Based Vesicles (Niosomes) in Drug Delivery. *Int J Pharm*.1998; 33.
31. Hunter CA.Vesicular System (Niosomes and Liposomes) for Delivery of Sodium Stribogluconate in Experimental Murine Visceral Leishmaniasis. *J Pharm. Pharmacol.* 1988; 161.
32. Nasseri B and Florence AT. Microtubules Formed by Capillary Extrusion and Fusion of Surfactant Vesicles. *Int J of Pharm.*2003; 91.
33. Ozer AY. A Novel Drug Delivery System Non -ionic Surfactant Vesicles. *Euro J Pharm Bio pharm.*1991; 75.
34. Bhaskaran S and Panigrahi L. Formulation and Evaluation of Niosomes using Different Non -ionic Surfactant. *Ind J Pharm Sci.* 2002; 63.
35. Arunothayanun P. The effect of Processing Variables on the Physical Characteristics of Non -ionic Surfactant Vesicles (Niosomes) formed from Hexadecyl Diglycerol ether. *Int J Pharm*. 2000; 7.
36. Stafford S, Ballie AJ and Florence AT. Drug effect on the Size of Chemically Defined Non -ionic Surfactant Vesicles. *J Pharm Pharmacol*.1988; 26.
37. Uchegbu IF and Florence AT. Non -Ionic Surfactant Vesicles (niosomes) Physical and Pharmaceutical chemistry. Adv. *Colloid Interface Sci.*1995; 1.
38. Manosroi A. Characterization of Vesicles Prepared with Various Non-ionic Surfactants mixed with Cholesterol. *Colloids and surfaces Biointerfaces*.2003; 129.
39. Balasubramaniam A. Formulation and In-Vivo Evaluation of Niosome Encapsulated Daunorubicin Hydrochloride. *Drug Dev and Ind Pharm*.2002; 1181.
40. Shahiwala A and Misra A. Studies in Topical Application of Niosomal Entrapped Nimesulide. *J Pharma Sci*. 2002; 220.
41. Baillie AJ. The preparation and Properties of Niosomes-Non -ionic Surfactant Vesicles. *J Pharm. Pharmacol*.1985; 863.
42. Schreier H., Liposomes and niosomes as topical drug carriers: dermal and transdermal delivery. *J. Controlled Release.*1985; 30: 863-868.

43. Buckton G., Harwood, Interfacial phenomena in Drug Delivery and Targeting Academic Publishers, Switzerland. 1995, 154-155.
44. Bairwa N. K., Choudhary Deepika., Proniosomes: A review, *Asian Journal of Biochemical and Pharmaceutical Research*. 2011; 2 (1): 690-694.
45. Suzuki K., Sokan K., the Application of Liposome's to Cosmetics. *Cosmetic and Toiletries.* 1990; 105: 65-78.
46. Chandraprakash KS, Udupa N., Uma Devi P., Pillai GK., Formulation and Evaluation of Methotrexate Niosomes. *Ind J Pharm.Sci.*1992; 54(5): 197.
47. V.C.Okore, A A.Attama, K.C.Ofokansl, C.O.Esimome1 and E.B.Onuigbo formulation and evaluation of niosomes. *Indian journal of pharmaceutical science,* 2011; 73, (3):323-328.
48. Bulto, T., F.H.Maskel,and, G.Fisseha.Skin Lesions in Resettled and Indigenous Populations in Gambela, with Special Emphasis on the Epidemiology of Tropical Ulcer. *"Ethiopian Medical Journal* 1993; 31: 75–82.
49. Carapetis, J. R., B. J. Currie, and E. L. Kaplan. 1999. "Epidemiology and Prevention of Group a Streptococcal Infection: Acute Respiratory Tract Infections, Skin Infections, and Their Sequelae at the Close of the 20th Century. *Clinical Infectious Diseases* 1999; 28: 205–10.
50. Daroczy, J. Antiseptic Efficacy of Local Disinfecting PovidoneIodine (Beta dine) Therapy in Chronic Wounds of Lymphedematous Patients. "Dermatology 2002; 204: 75–78.
51. Eells, L. D., P. M. Mertz, Y. Piovanetti, G. M. Pekoe, and W. H. Eaglestein.. "Topical Antibiotic Treatment of Impetigo with Mupirocin." *Archives of Dermatology* 1986; 122: 1273–76.
52. Lopez-Gomez, S., A. Del Palacio, J. Van Cutsem, M. Soledad Cuetara, L. Iglesias, and A. Rodriguez-Noriega. 1994. "Itraconazole versus Griseofulvin in the Treatment of Tinea Capitis: A Double-Blind Randomized Study in Children." *International Journal of Dermatology* 1994; 33: 743–47.
53. Baan siddhi J, Pechaaply D, 1988. Botanical report of some Thai medicinal plants, part I, Bangkok: *Department of Medical Sciences.*1988.; p.8-9.
54. Ali-Emmanuel NM, Moudachirou AJ, Akakpo J, Quetin-Leclercq Activities in vitro antibacterial Cassia alata, Lantana camara and Mitracarpus scaber on

Dermatophilus congolensis isolated in Benin. *Revue Élev. Méd. V ét. Pays Trop.,*2003; 55: 183-187

55. Anandan R, Jayakar B, Manavalan R, Hepatoprotective Activity of the Infusion of the Dried Leaves of Cassia Alata Linn. Biomed. *Pharmacol. J*.; 2009; 2(1):113-116.
56. Archana P, Samantha T, Mahitha B, Chamundeswari, Rama SN Preliminary phytochemical screening from leaf and seed extracts of Senna alata. Roxb-an Ethno medicinal plant. *International Journal of Pharmaceutical and Biological Research* .2012; 3(3): 82-89.
57. Blytt HJ, Guscar TK Butler LG Ant nutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. *J Chem Ecol,* 1988; 14: 1455-1465.
58. Dakuyo ZP, Sekhoacha MP, Matsabisa MG, Nikièma JB.,Ouedraogo JB, Ouedraogo GA.Evaluation of the antiplasmodial activity and lethality of the leaf extract of Cassia alata. (Fabaceae). *Pak. J. Biol.Sci.,* 2016; 19: 171-178.2016.
59. Demirezer LO, Kuruüzüm-Uz A, Bergere I, Schiewe HJ, Zeeck A,The structures of antioxidant and cytotoxic agents from natural source: Anthraquinones and tannins from roots of Rumex patientia. *Phytochemistry,* 2001; 58: 1213-1217.
60. Doughari JH, Okafor B, Antimicrobial activity of Senna alata Linn*. East and Central African Journal of Pharmaceutical Sciences*, 2007; 1: 1721.
61. Effraim KD, Sodipo OA, Jacks TW, Antihepatotoxic activity of aqueous of Cassia alata (Linn) leaves against carbon tetrachloride induced liver damage in rats*. Pakistan Vet J*. 1999; (3):111-114.
62. Federicci E, Palazzino G, Nicoletti M, Caleffi C, Antiplasmodial activity of the alkaloids of Paschiera fuchsia folia. Plantae. Med. 2000; 66(1): 93-95.
63. Ibrahim D, Osman H, Antimicrobial activity of Cassia alata from Malaysia. *J Ethnopharmacol,* 1995; 45(3), 151-156.
64. Igwe OU, Onwu FK, Leaf essential oil of Senna alata Linn from South East Nigeria and its antimicrobial activity. *International Journal of Research in Pharmacy and Chemistry.* 2015;5(1) 27-33;

65. Jyothirmai M, Himavysnavi K, Himabindu N, Bhargavi C, Ravi KA, Chinna EM Phytochemical evaluation of Cassia alata, Techolospermum jasminoides and Caesalpiniaceae sappan.*Indian Journal of Research in Pharmacy and Biotechnology,* 2015 ; 3(6) : 467

66. Kayembe JS, Taba KM, Ntumba K, Kazadi TK, *In vitro* Antimalarial Activity of 11 Terpenes Isolated from Ocimum gratissimum and Cassia alata Leaves. Screening of Their Binding Affinity with Haemin*. Journal of Plant Studies*; 2012; 1 (2): 168-172.

67. Khare CP (2007). Indian Medicinal Plants: An Illustrated Dictionary. *Springer-Verlag Berlin Heidelberg London* 2007; 126-133.

68. Lewis A, Levy A, Anti-inflammatory activities of Cassia alata extract in Complete Freund's adjuvant arthritis in rats. *West Indian Med J.* 2011; 60(6): 615-621.

69. Neil Alejandro A. Pinarok, Lylah Daisy A. Acorin, Delbert A. Dala, Jenny Rose U. Kruse, Maricar T. Obina, Loida A. Rapada, Debbie Joyce R. Voloso, Rey Niel C. Salac, Aljon Victor Nibalvos Development of Cassia alata Linn. (Akapulko) ointment using Jatropha curcas Linn. (tuba-tuba) oil as base. *International Journal of Advanced Scientific and Technical Research* 2017; (7)259-276.

70. Vigbedor Bright Yaw, Osafo Acquah Samuel, Ben Adu Gyan B, Lotsi Bertha In vitro antimalarial activity of the ethanol extracts of Afzelia Africana and Cassia alata commonly used as herbal remedies for malaria in Ghana *International Journal of Novel Research in Life Sciences*, 2015;(6):10-16.

71. Raphael M. Mordi; Amalawa N. Osula; Clara L. Igeleke; Emmanuel E. Odjadjare; F. O. J. Oboh; Esosa O Uwadiae The evaluation of the antimicrobial property of Cassia alata leaves in Benin City, Nigeria Department of Basic Sciences., *Biomedicine and Nursing* 2016;2(3) 14-23

72. Addai Mensah Donkor Richard Mosobil Jennifer suurbaar In Vitro Bacteriostatic and Bactericidal Activities of Senna alata, Ricinus communis and Lannea barteri extracts Against Wound and Skin Disease Causing Bacteria *Journal of Analytical & Pharmaceutical Research J Anal Pharm Res* 2016; 3(1): 00046

73. Senga Kitumbe , Nkanga Isalomboto , Ngoro Vero, Welo Unya , Opota Onya Formulation Of A Herbal Dermal Ointment Using Whole Leaves Of Cassia Alata Linn. *International Journal of Ayurvedic and Herbal Medicine,*2016;6: 2355–2363

74. M. S.Rahman, M. S. Yeasmin M. N. Begum studies on the isolation of 2, 5, 7, 4′ ″ tetrahydroxy isoflavone from the leaves of cassia alata, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*.2015; 3(2):64 - 70.

75. Victor Arokia Doss, Mohanasundaram Sugumar and Prasad Maddisetty, P.N., Bioactive compounds with multiple therapeutic values of Senna alata (l.), an ornamental shrub from the botanical family of Fabaceae *International Journal of Current Research,* 2015; 7,12: 23892-23896

76. J. Meenu priya, A. Sahaya Vinisha and P. Priya Phytochemical screening and bioassay of Cassia alata leaf extract to study its skin hyperpigmentation activity *World Journal of Pharmaceutical Sciences,*2014;2321-3310;

77. Rama Swamy Nanna, Archana Pamulaparthi, Vamshi Ramana Prathap, Mahitha Banala experimental Evaluation of anti-diabetic activity and anti hyperlipidemic evaluation of leaf extracts of senna alata in alloxan induced diabetic rats *European Journal of Pharmaceutical and Medical Research* 2015;2(4):227-237.

78. Pratiwi Wikaningtyas and Elin Yulinah Sukandar The antibacterial activity study of senna alata leaf extract and fraction towards mrsa (methicillin resistant staphylococcus aureus) and its mode of action *World Journal of Pharmacy and Pharmaceutical Sciences,* 2015; 126-133.

79. Mulham alfatama, kausar Ahmad, farahidah moham microencapsulation of cassia alata: fabrication and characterization ED *International Journal of Pharmacy and Pharmaceutical Sciences,* 2012; 0975-1491.

80. Selvi V, I. Isaivani and S. Karpagam Studies on antimicrobial activities from flower extract of Cassia alata Linn. Int J CURR SCI 2012; 299- 303.

81. Silver Ighosotu and Nyerhovwo J, Tonukari., the effects of Cassia alata L. aqueous leaf extract on the germination of Corchorus olitorius *Journal of Agricultural Biotechnology and Sustainable Development* 2013; 5(1),1-5.

82. Mohammed I. Ali, Ahmed M. Aboul-Enein, Samy M. Mohamed, Faten M. Abou elella, Magdy M. D. Mohammed, Ahmed R. Hamed., Phytochemical,

cytotoxicity and antioxidant investigation of Cassia alata leaves growing in Egypt, *Journal of Innovations in Pharmaceutical and Biological Sciences* (JIPBS) 2017 ; 2349-2759.

83. R.P.Senthil kumar, V. Malaya man and S. Sindhuja Phytochemical screening and antibacterial evaluation of the leaf, flower and seed coat extracts of Cassia alata L, *Journal of Chemical and Pharmaceutical Research*,2013;5(11):740-744
84. Panichayupakaranant, P. and Kaewsuwan, S. Bioassay-guided isolation of the antioxidant constituent from Cassia alata L. leaves Songklanakarin *J. Sci. Technol.*, 2004; 26(1): 103-107.
85. Da FL, Keugni, Belemtougri, Fotio, and Dimo. Protective Effect of Dichloromethane Extract of Cassia alata (Linn.) Leaves on Complete Freund's Adjuvant-Induced Inflammation in Rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2018; 229-238.
86. Ikechukwu O. Agbagwa Crude Extracts of Senna alata (L.) Roxb. Mimics Plant Growth Hormones in Promotion of Vegetative and Reproductive Growth in Celosia argentea L *American Journal of Plant Sciences*, 2014; 1919-1925.
87. Chris. A. Alalor, Cecilia. Igwilo, Chukwuemeka. Azubuik Evaluation of the Antibacterial activity of Herbal ointments formulated with Methanolic extract of Cassia alata L, *Asian Journal of Biomedical and Pharmaceutical Sciences* 2012;15-19.
88. Okooboh, Asotie Matthew, Gambo, Nanbol Ndam Phytochemistry and Antimicrobial Activity of the Leaf of cassia Alata linn. *Chemistry and Materials Research* ,2013;96-101
89. Adlis Santoni, Mai Efdi and Ismail Structure Elucidation of Anthraquinone Derivate from Cassia Alata Linn and Antioxidant Activity Journal of Chemical and Pharmaceutical Research, 2016; 8(10):117-120
90. Douye V. Zige Elijah I. Ohimain Biobelemoye Nengimonyo Antimicrobial Activity of Ethanol Extract of Senna Alata Leaves against Some Selected Microorganism. *Greener Journal of Microbiology and Antimicrobials,* 2014; 26-31.

91. K. kavipriya and M. chandran FTIR and GCMS Analysis of Bioactive Phytocompounds in Methanolic Leaf Extract of Cassia Alata *Biomedical & Pharmacology Journal ,* 2018; 11(1):141-147

92. Kudatarkar NM and Nayak YK Pharmacological Screening of Cassia alata leaves on Colorectal Cancer *iMedPub Journals*, 2018; 1-7.

93. Solomon Oluwole Oladeji, Funmilayo Enitan Adelowo and Kehinde Abraham Ode lade Mass spectroscopic and phytochemical screening of phenolic compounds in the leaf extract of Senna alata (L.) Roxb. *Brazilian Journal of Biological Sciences,* 2016;210-219.

94. G.R.Nalini, Azad S.A.K, Cherryl D.M Documentation and Comparison of Antifungal Sensitivity of Cassia alata Leaf and Flower Extracts against Candida albicans *International Journal of Pharmacy and Pharmaceutical Sciences* ,2017; 192-197.

95. Mitra Prasenjit, Ghosh Tanaya, Gupta Sumanta, Basu Basudeb and Mitra Prasanta Kumar,Isolation and Characterization of a Compound from the Leaves of Cassia Alata Linn. ECRONICON open acess chemistry 2016;2(2):138-144

96. M. Wuthi-udomlert S. Prathanturarug N. Soonthornchareonnon, Antifungal Activities of Senna alata extracts Using Different Methods of Extraction Act a Hort. 2003;597.

97. Timothy SY, Wazis CH, Adati RG and Maspalma ID Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of Cassia Alata Linn Journal of Applied Pharmaceutical Science, 2012; 02 (07); 182-185.

98. Oke D. G., Oluranti O. O. and Akande J. a Preliminary studies on the laxative properties of Senna alata L. and Hollandia yoghurt, *African Journal of Biotechnology,* 2018; 431-432.

99. Mansuang Wuthi-udomlert, Pavena Kupittayanant and Wandee Gritsanapan in vitro evaluation of antifungal activity of anthraquinone derivatives of senna alata J. *Healtb Res*, 2010;24(3) 117-122.

100. Funmilayo Adelowo, Oluwole Oladeji an Overview of the Phytochemical Analysis of Bioactive Compounds in Senna alata , *Advances in Biochemistry* ,2017; 5(5):102-109.

101. U. Igweand FK. Onwu leaf essential oil of senna alata linn from south east Nigeria and its antimicrobial activity international journal of research in pharmacy and chemistry IJRPC 2015, 5(1), 27-33

102. R.M. Kolawole, B.T. Thomas, J.B. Folorunso and A. Oluwadun GC-MS Analysis and Antifungal Activity of Senna alata Linn *,International Journal of Microbiological Research* ,2015;6 (2): 100-107.

103. S.Mohideen, e.sasikala, and p.aruh.aj, Pharmacognosy of Cassia Alata Linn – leaves *ancient life of science*, 2005; 192-198.

104. Pharkphoom Panichayupakaranant, Apirak Sakunpak, and Athip Sakunphueak Quantitative HPLC Determination and Extraction of Anthraquinones in Senna alata Leaves *Journal of Chromatographic Science*, 2009; 47.

105. Simplice Joel Ndendoung tatsim, jean-de-dieu tamokou, virginie tankeu tsague, marc lamsho, prodipta sarkar, Prasanta kumar bar and Michael Spitteler., Antibacterial-guided isolation of constituents from Senna alata leaves with a particular reference against Multi-Drug-Resistant Vibrio cholera and Shigella Flexner*. International journal of biological and chemical sciences,* 2017; 46-53.

106. Olivier Tene Tcheghebe, Viviane Raïssa, Francis Ngouafong, Tatong Kamsu, Tchuente Ethno botanical uses phytochemical and pharmacological profiles, and toxicity of Cassia alata L. *Medicine and Medical Sciences* 2017;4(2):16-24.

107. P.l.rajagopal, majetha,. k.r.sreejith, s.s. Kiron, s.aneeshiad, dr.k.premaletha phytopharmacological review on cassia alata linn *journal of international academic research for multidisciplinary impact factor* ,2014;1 .393, 2320-5083.

108. CA Alalor C.I. Igwilo and E.jeroh journal of pharmacy and allied health science evaluation of the antibacterial properties of aqueous and methanolic extracts of cassia alata*, journal of pharmacy and allied health science* 2012; 16-24.

109. Onochie.I.T.O 1, Nwakile C.D UmeyorC.E, UronnachiE.M. OsonwaU.E Attama. A. and EsiomeC.O Formulation and evaluation of niosomes of Benzyl penicillin. *Journal of applied Pharmaceutical science*, 2013; 3 (12): 066-071.

110. Katare O.P (2001) prepared and in vitro evaluated the liposomal and niosomal delivery system for ant psoriatic drug dithranol, *International Journal of Pharmaceutics* ,2001;228,43-52.

111. Jia - you. Fang. Effect of the skin permeation and partitioning of antibacterial agent enoxacin in liposomes and niosomes, *International Journal of Pharmaceutics* 2001

112. Anna m.fadda niosomes carries for Tretinoin III. Study into the in vitro cutaneous delivery of vesicle incorporated Tretinoin; *international journal of pharmaceutics,* 2006; 311: 11-19.

113. Ibrahim. A. Alsarra, proniosomes as a drug carrier for transdermal delivery of ketorolac*; European Journal of pharmaceutics and Bio pharmaceutics*, 2005;59: 485 – 490.

114. Yi.Hung Tsai Niosome suspension and proniosomes gel showed different behaviour in modulating transdermal delivery of estradiol across the skin.2000;3(2) 1-9.

115. H. M. shreedevi, J. Adlin Jino Nesalin, T. Tamizh Mani. Development and evaluation of stavudine niosome by ether injection method. International Journal of Pharma Sciences and Research, 2016; 7 (1) : 0975-9492.,

116. Ahmad Usama, Gihan Fetih, and Tahani, EL-Faham, Performance of meloxicam Niosomal gel Formulations for Transdermal Drug Delivery .*British Journal of Pharmaceutical research,* 2016;12(2): 1-14,

117. Tamizharasi S, Dubey A, Rathi V ,Rathi JC, Development and characterization of Niosomal Drug Delivery of Gliclazide. J young pharm. 2009; 1(3):205-209.

118. P.Aravind Kumar, Ranjit Singh, K. Karthick and K.S.G. Arulkumaran. Formulation and evaluation of sustained released niosomes containing pregabalin*. International Journal of Pharmacy & Life sciences,* 2013; 4 (7): 2770- 2774.

119. Ranga Priya M, Natrajan R, and Rajendran NN. Formulation and Evaluation of Zidovudine Loaded Niosomes. Research and Reviews: *Journal of pharmaceutics and nanotechnology,* 2013.1(1): 12- 18.

120. Naresh Ahuja, Vipin Saini, Vijay kumar Bishnoi, Atul Garg, Monika Hisoria Joyati Sharma and kunul nepali. Formulation and Evaluation of Lansoprazole niosome, 2008; 1 (3): 561-563.
121. Alok kumar Srivasta v, Priyanka Das, To study the Formulation of Niosome of Ofloxacin and its Evaluation for Efficacy Anti-Microbial Activity *International Journal of Innovative Research in Science, Engineering and Technology,* 2014; 3(12).
122. Agrawal, V., Desai, S. centrifugally accelerated thin layer chromatography for isolation of marker compounds and bioactive, *Journal of Pharmacognosy and Phytochemistry,* 2015;3(6):145- 149.
123. Ahmed, F & Urooj, A. Traditional uses, medicinal properties, and phytopharmacological of Senna alata: A review, Pharmaceutical Biology, Information Healthcare, Pharmaceutical biology, 2010; 48(6):672-681.
124. Fans worth and Bunyapraphatsara Thai ethno medical text book 1992; 45-58.
125. Lubrizol, carbopol controlled release drug development, Ind, pharm, 2006; 32 : 95-106.
126. Yusef., study of topical application of carbopol 1993;1-15.
127. Liu et al., 2008 advantages of carbopol 2008;4(2):24-30
128. Prepared at the 28th JECFA published in 1984;31/2 : 52.
129. Metals and arsenic specifications revised at the 55th JECFA,2000;
130. Z. Markovi,N. Manojlovi, and S. Zlatanovi. Electronic Absorption Spectra of Substituted Anthraquinones and Their Simulation Using ZINDO/S Method, *Journal of the Serbian Society for Computational Mechanics,* 2008; 2:73-79.