Pharmaceutical Development and Evaluation of Mamajjaka Ghanavati

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHARMACY (AYURVEDA)

In

Rasashastra & Bhaishajya Kalpana

By

Shivangni Raj
11501454

Under the guidance of

Dr. Manish Vyas
Associate Professor
Rashashastra & Bhaishajya Kalpana

School of Ayurvedic Pharmaceutical Sciences
Lovely Professional University
Punjab 144411
November
2017

Statementby the Candidate

This is to submit that this written submission in my project report entitled **"Pharmaceutical development and evaluation of Mamajjaka Ghanavati"** represents original ideas in my own words and where others 'ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above wills because for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required. I assure and hold full responsibility for its genuineness.

Forwarded Through
Dr. ManishVyas
Associate Professor

Shivangni Raj
Reg. No: 11501454

Certificate by Supervisor

The work described in this project report entitled **"Pharmaceutical development and evaluation of Mamajjaka Ghanavati"** has been carried out by **Shivangni Raj (11501454)** under my supervision. I certify that this is her bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Date:
Place:

Dr. Manish Vyas
Associate professor

Certificate by School

This is certified that the work described in this project report entitled **"Pharmaceutical development and evaluation of Mamajjaka Ghanavati"** Has been carried out by **Shivangni Raj** at the School of Ayurvedic Pharmaceutical Sciences, Lovely Professional University, Punjab.

Mr. Saurabh Singh Baghel
(COD)
Ayurvedic Pharmacy

Dr. Monica Gulati
(Professor & Sr. Dean)
Sr. Dean and Head of School

Acknowledgement

First and above all, I praise god, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to assistance and guidance of several people.

I would like to express my deepest gratitude to my guide **"Dr. Manish Vyas" Associate professor**, Lovely School of Ayurvedic Pharmaceutical Sciences, Lovely Professional University for their valuable warm encouragement, thoughtful guidance, gracious attention and motivation for completing my project work. It gives me immense pleasure to submit my dissertation work as his student.

I want to express my deep thanks to my **Co-Guide "Dr. Gopal Lal Khatik**" for the trust, the insightful discussion, offering valuable advice, for your supportduring the whole period of the study, and especially for your patience and guidance during the writing process.

I am very obliged to **"Mr. Saurabh Singh Baghel"** HOD Lovely School of Ayurvedic Pharmaceutical Sciences, Lovely Professional University for giving his support to proceed my project work.

I am very grateful to **"Dr. Monica Gulati"** Senior Dean Lovely School of Applied Medical Sciences, Lovely Professional University whose continuous guidance helped me a lot to face many problems during my project work.

I would like to thank my parents **"Mr. Deepak Raj"** and **"Mrs. Uma Raj"** who have always been with me. It was due to my parent's dream, ambition, and sacrifice that I get so much ability to face all challenges during my research work today. I would like to express my heartiest gratitude to my friends **"Anil Kumar Sah, Neha Bhatia, Saveena Chauhan, Arun Kumar, Swati Sharma, Sweta pathyarach, Ambika Thakur, and Preeti Kalsi"** as without their love and support it would not have been possible to complete the task.

Words will fall short to express my feelings for my parents and my guide. They are the soul of my energy and motivation. Their blessings have tolead me towards achieving my goals.

INDEX

LIST OF TABLES

LIST OF ABBREVIATIONS

Ch. Chi.	– Charakchikistasthan
Su. Su.	– Shusrut sutra sthana
A.P.	– Ayurvedprakash
Sha. Sam. Ut.Kha.	– Sharangdharsamhitautar khan
mg.	– Milligram
g.	– Gram
mm.	– Millimeter
ml.	– Millilitter
Kg.	– Killogram
Dia.	– Diameter
µg	– Microgram
Conc.	– Concentration
Abs.	– Absorption
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
Charac.	– Characteristics
SNEDDS	– Self emulsifying drug delivery system
IC_{50}	– Inhibitory concentration
hrs.	– Hours
nm	– Nanometer

ABSTRACT

Ethnomedicine is contributed to the evolution of different systems of medicine viz. Ayurveda, Siddha, Unani, Naturopathy including the modern medicine. It has continuously providing the information about the various effective drugs for the exploration of their therapeutic profile**.** *Enicostemma littorale* is one of the traditional drugs used mainly in Gujarat, Madhya Pradesh, and Rajasthan as a stomachic, tonic, carminative and appetizer. It is generally prescribed in the form of vati for the treatment of type 2 diabetes in Ayurveda. Recent studies based on the anti-diabetic effect of mamajjaka suggesting its role to reduce blood glucose and increase serum insulin level. However, standards of mamajjaka ghanavati are not available in the official monographs. So, present study was designed to develop standards for the preparation and evaluation of mamajjaka ghanavati. The average yield of ghana was 23.75%. The dose of prepared vati was 500 mg. Vati was complying with the various standards including disintegration, hardness, weight variation, and friability. HPTLC profile revealed the presence of 9 constituents in ghanavati. In addition to this, SNEDDS were also prepared to improve the efficacy of the mamajjaka. Labrafil, tween-80 and Transcutol P were used as oil, surfactant, and co-surfactant respectively for the preparation of SNEDDS. Nano droplet size and zeta potential of SNEDDS were optimized, noted as 500nm and -22.8 mV respectively. Entrapment efficacy of prepared formulation was 92%. *In-vitro* antioxidant and anti diabetic activities of mamajjaka ghanavati and SNEDDS revealed that nano formulation is the better dosage form for the mamajjaka than ghanavati.

CHAPTER 1

INTRODUCTION

Ethnomedicine is the knowledge based on the curative and palliative effects of certain herbs, animals and minerals. This knowledge is the outcome of trial and error practices of the several generations. Ethnomedicine is contributed to the evolution of different systems of medicine viz. Ayurveda, Siddha, Unani, Naturopathy including modern medicine. It has continuously providing the information about the various effective drugs for the exploration of their therapeutic profile**.** *Enicostemma littorale* is one of the traditional medicine used mainly in Gujarat, Madhya Pradesh, and Rajasthan as a stomachic, tonic, carminative[1] and appetizer. *E. littorale* is also prescribed as a single or in combination in the form of *Vati* (Pills) for the treatment of type 2 diabetes in Ayurveda. Recent studies based on the anti-diabetic effect of *E. littorale* suggesting its role to reduce blood glucose and increase serum insulin level. Significant improvement in kidney function, lipid profile, systolic and diastolic blood pressure also reported[2]. Moreover, it possesses multidimensional therapeutic properties viz. antimicrobial activity,[3] antihelminthic activity,[4] antinociceptive effect,[5] antioxidant activity,[6] antiulcer activity,[7] anti-inflammatory activity, antitumour activity,[8] hepatoprotective activity,[9] hepatomodulatory activity, and antihyperlipidaemic activity including the hypoglycemic activity,[10] antihyperinsulinemic activity, and aiabteic neuropathy activity.

WHO (World Health Organization) and Ministry of AYUSH, India collaboratively establishing protocols or monographs for the global acceptance of traditional medicines. Standardization confirms the identity, quality, purity and efficacy of drugs and formulations.[11] Therefore, continuous efforts of APC (Ayurvedic Pharmacopeia Committee) & PLIM (Pharmacopoeial Laboratory for Indian Medicines) have resulted in the publication of several monographs for the standardization of ayurvedic drugs and formulations. However, standards are not available for the mamajjaka ghanavati which commonly used by the traditional healers and ayurvedic physicians for the treatment of diabetes. Hence, the present study has been designed to prepare and standardize the mamajjaka ghanavati by using different parameters and further evaluated for its anti-oxidant and antidiabetic activities by using *in-vitro* models.

CHAPTER 2

TERMINOLOGY

Standardization Standardization of drug is defined as authentication of its identity, determination of quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological evaluations.

Vati and gutika Classical solid and unit dosage form similar like pills are known as vati and gutika.

Ghanavati Ghanavati is pill form of ghana.

SNEDDS Self nano emulsifying drug delivery system.

Characterization The art of describing the character.

CHAPTER 3

3.1LITERATURE REVIEW OF GHANA AND GHANAVATI

3.1.1 Samhita Kala: Various references are available regarding raskriya in Charaka and Sushruta samhita which can be compared with ghana kalpana.

Classical text	Description	Reference
Charak Samhita[12]	Ghana is 1st time mentioned in charaksamhita. Charak has mentioned the ghana kalpana according to its method of preparation and consistency like dadhi (Curd)	Ch. Chi. 15/229
Sushruta Samhita[13]	Acharya Dalhana defined raskriya. In raskriya decoction is prepared from 8 or 16 times of water and evaporated till 1/8 or 1/16 parts of water remain. Then, subjected to further heating till it becomes thick.	Su. Su. 37/21 (Dalhana Tika)

Table 3.1: Description available in samhitakala

3.1.2 Formulations mentioned in Charaksamhita:

S.No.	Formulations	References
1	Dravyadiraskriya[14]	Ch. Chi. 26/202
2	Pippalyaadi raskriya[15]	Ch. Chi. 26/258
3	Krishnasarparasadi raskriya[15]	Ch. Chi. 26/259
4	Dhatryadi raskriya[16]	Ch. Chi 26/260-261

Table 3.2: Rasakriya mentioned in Charaka samhita

3.1.3 Sangraha Kala:

Various references are available in Astanga sangraha, Bhavaprakash, Ayurveda prakash and Sharangdhara samhita which can be correlated with Ghana kalpana.

Classical text	Description
Astanga Sangraha[17]	Rasakriya is one of the type of anjana kalpana.
Bhavaprakash[18]	Description about rasanjana is available. Decoction of darvi added with an equal quantity of milk and heated till it becomes thick termed as rasakriya.
Ayurveda Prakash	The method of preparation of rasanjana with aja milk.
Sharangadhara Samhita[19]	Examples of rasakriya under anjana kalpana is mentioned i.e. davryadi rasakriya, babul rasakariya.

Table 3.3: Description available in Sangraha kala

3.1.4 Adhunik Kala (Sidha Yoga Samgraha):

Guduchi ghanavati is mentioned by Acharya Yadavaji Trikamaji as Samshani Vati in Jwaradhikar.[20]

3.1.5 Ghana

Ghana is a concentrated form of the liquid material of plant like swarasa and decoction etc. Ghana is actually a dried aqueous extract. The liquid content of 'kwatha' is evaporated with the help of heat. In ayurvedic literature, the Raskriya is considered as Ghana, Phanita, Avleha because the method of preparation of these dosage forms is similar except minor differences. Avleha is semi-solid which can be licked whereas 'Ghana' is a solid form.

3.1.6 Definition of Ghana

Swarasa and kwatha of plant parts when further heated to concentrate is known as rasakriya.[21]

"Aqueous solutions of plants i.e. swarasa and kwatha when concentrated with the help of heat to get the semi-solid form is termed as Ghana."

3.1.7 Synonyms of Ghana

There is no synonyms mentioned for Ghana but due to the common pharmaceutical procedure of Leha, alveha, and phanita,[21] has mentioned as synonyms of raskriya by Acharya Sharangdhara. All these terms have their specific meaning and therapeutical value. Avleha and khanda are concentrated form liquids with sweet substance but Ghana and raskriya are concentrated forms of aqueous solution without sweet substance.

3.1.8 Advantages of Ghana:[22]

- Highly concentrated.
- Reduced dose.
- More shelf life.
- More stable.
- Increased bioavailability.

3.1.9 Ghana Vati

Ghanavati is compressed pill form of ghana. Many formulations of ghanavati are available in classical texts like sarpagandha ghanavati and guduchi ghanavati. In charaksamhita it is mentioned that vati is prepared from semisolid form.[23]

3.1.10 Vati kalpana:

The pill form of the medicines is a convenient form for the patient as well as a physician in treatment[24]. Vati is made in the shape of flat circular mass hence it is similar to the pills.

3.1.11 Definition: Medicines prepared in the form of tablet or pills are known as Vati or Gutika.[25]

3.1.12 Synonyms of vati: Gutika, vati, vatika, modak, pindi, guda and varti.

3.1.13 Types of vati kalpana:

1. **Agnisadhya Vati:** Vati is prepared with the help of heat. The sugar or jiggery or guggulu is prepared like leha on mild heat and powder materials are added to leha and then vati is made by rolling, circular in shape
2. **Anagnisadhya Vati:** Vati is made without heat. Powder materials are triturated with guggulu, guda or suggested liquid or to make thevati.[26]

3.1.14 Dose of ghanavati:

The dose of ghana is not clearly mentioned in our classics but, there is reference regarding Samsamani Vati in Siddha Yoga Sangraha. The dose of ghana is mentioned as 5 to 10 Vati of 2 Ratti (250 -500mg) four to five times a day. So, the dose of ghana may be taken 5- 10 g per day.[27]

3.1.15 Some formulations of ghanavati with references:[25]

S. No.	Formulation	Reference
1	Kutajaghana vati	Siddhayogasangraha, atisara-pravahika-grahanyadhikara
2	Sarpagandhaghana vati	Siddhayogasangraha, bhrama-anidra-unmadadhikara
3	Sarpagandhaghana Vati	Siddhayogasangraha, bhrama-Anidra-unmadadhikara
4	Samsamani Vati (Guduci Ghana Vati)	Siddayogasangraha, jvaradhikara

Table 3.4: Examples of ghanavati with reference

3.2 Literature review of SNEDDS

3.2.1 SNEDDS

It is an isotropic mixture of oil, surfactant and co-surfactant having a specific ability to develop oil-in-water (O/W) nano-emulsion in aqueous media.[28]

3.2.2 Types of nano-emulsion (SNEDDS)

3.2.2.1 Water in oil (W/O)

Droplets of water dispersed in continuous phase oil.

3.2.2.2 Oil in water (O/W)

Droplets of oil dispersed in continuous phase water.[29]

3.2.2.3 Biphasic nano-emulsion

Surfactant soluble in oil as well as water phase and dispersion of droplet in both i.e. oil and water phase.[30]

3.2.3 Selection of excipients for SNEDDS

Self-emulsification is depending upon the nature and concentration of oil, surfactant and co-surfactant. Very few excipients are having the property to develop good self-emulsifying system.[31]

3.2.4 Excipients used in formulation of SNEDDS

3.2.4.1 Oils

Oil is very important excipient for the preparation of SNEDDS because it helps in emulsification and increases the lipophilicity via intestinal lymphatic system. Oils help to enhance the absorption in GI tract depending upon nature of triglyceride.[32] Synthetic or chemically modified oils are extensively used to prepare self-nanoemulsion such as hydrolyzed vegetable oils which is having better ability to form self-emulsification because they also hold surfactant property.[33,34]

3.2.4.2 Surfactants:

Surfactants are defined as amphiphilic molecules and having of both hydrophilic and lipophilic or hydrophobic parts. The function of surfactants is to enhance self-emulsification ability of SNEDDS, which further improves the bioavailability of partially absorbable drugs.[35]

3.2.4.3 Co-surfactants:

Co-surfactants like mono ethyl ether (transcutol P), diethylene glycol, poly ethylene glycol, ethanol, capyrol 90, poly- oxyethylene, propylene carbonate, lauroglycol, propylene glycol, glycofurol and spans can increase the solubility of high amounts of hydrophilic surfactants or hydrophobic drugs in the lipid or oil base.[36]

3.2.5 Mechanism of self‑emulsification

In the process of emulsification, the associated free energy (ΔG) is can be explained by following equation.

$$G = \Sigma N\pi r2\sigma$$

Where**,**

ΔG = free energy associated with the process

N = number of droplets

r = Radius of droplets

σ = interfacial energy

It is apparent from the above equation that the spontaneous formation of the interface between the oil and water phases is energetically not favored. The system commonly classified as SEDDS has not yet been shown to emulsify spontaneously in the thermodynamic sense. The emulsification process may be associated with the ease with which water penetrates the oil – water interface with the formation of liquid crystalline phases resulting in swelling at the interface, thereby resulting in greater ease of emulsification. [37]

3.2.6 Characterization of SNEDDS

Entrapment efficiency, particle size, poly-disperse index, zeta potential, and TEM are generally used for the characterization of SNEDDS.

3.2.7 Advantages of SNEDDS

SNEDDS are novel approach in order to improve the bio-availability of lipophilic drugs administered through the oral route. It has many benefits over the conventional emulsions including the long-term stability, patient compliance, palatability, ease of manufacture & scale-up, and quick onset of action**.** [38,39]

3.3 LITERATURE REVIEW OF DRUG

3.3.1Mamajjaka (*Enicostemma litorale* Blume)

This plant is first time mentioned in Shodhal nighantu (12th century) in lakshamanadivarg. It is a traditional popular herb used in the treatment of diabetes mellitus.[40] This plant is also used in combination with other drugs.[41]Number of polyherbal formulations are available in the market in which mamajjaka is used as an important ingredient like Diasol, Dihar.[42] Mamajjaka ghanavati i.e. pill form of ghana used for treating the type-2 diabetes by reducing blood glucose and increase serum insulin level.

3.3.2 References found in Nighantus about Mamajjaka:[43]

S. No.	Nighantu	Period	Varga
1.	Sodhala Nighantu	12th century AD	Lakshamandi varga
2.	Saligram Nighantu	19th century AD	Parishishta bhaga
3.	Nighantu Adarsh	20thcentuary AD	Kiratadi gavar
4.	Priya Nighantu	20th century AD	Satapushpadi varga

Table 3.5: Mamajjaka in Nighantus

3.3.3 Taxonomic position of *Enicostemma littorale*:[44]

Kingdom **:** Plantae
Subdivision **:** Angiospermae
Class **:** Dicotyledonae
Subclass **:** Gamopetalae
Series **:** Bicarpellatae
Order **:** Gentianales
Family **:** Gentianaceae
Genus **:** *Enicostemma*
Species **:** *littorale*

3.3.4 Vernacular name:[44]

Sanskrit : Mamajjaka, Naahi, Tikshanpatra
Hindi : Naahi, Chhota Chirayata
Gujarati : Mamejavo

Bengal : Nagajivha

Tamil : Vellarugu or Vallari

English name : White head, Indian Gentian

3.3.5 Ayurvedic properties of mamajjaka[45]

Rasa : Tikta

Guna : Laghu, Ruksha

Virya : Ushna

Vipaka : Katu

Doshakarma : Kaphapittashamak

3.3.6 Description of plant:

Enicostemma littorale Blume (Mamajjaka) is a glabrous perennial herb[45] attaining a height of 5-20 inches, distributed throughout India up to a height of 1500 feet. Drug consist of dried whole plant of E. littorale Blume (Enicostemma hyssopifolium) belonging to the family Gentianaceae.

Figure: 3.1 Habitat of *Enicostemma littorale* Blume.

3.3.6.1 Macroscopic characteristics

Stem is cylindrical, glabrous, readily rooting at nodes; no odour; taste-bitter.

Leaves are sessile, longer than internodes; 5-8× 0.3-1 cm. Midrib depressed on adaxial side.

Flowers are white in colour arranged in a cluster, numerous in the axils of each pair of leaves. Calyx tube 1-2 mm, unequal lobes 0.7-1.5× 0.4-0.7 mm. Corolla tube 3.5-6mm.

Fruits are capsule, globes with pale, ridged ovate seeds 0.4-0.5mm in diameter. This plant is used as a folk medicine in the treatment of inflammation, diabetes mellitus and to regulate bowel functions[46] in western and southern India.

Roots are 2-4mm in dia., taproot dull white, surface slightly rugose.

Figure: 3.2 Leaves, flowers, and stem of *Enicostemma littorale* Blume.

3.3.6.2 Microscopic characteristics

Leaf- Presence of prominent bulge abaxially in transverse section of the midrib of the leaf, collenchymas cells, vascular bundle, and parenchyma cells in ground tissues. Epidermis is single layered, papillae on the epidermis, anisocytic stomata.

Stem-Quadrangular stem and having narrow wings, single layered epidermis, collenchymas and parenchyma are present in winged corners, uniseriate medullary rays, presence of starch grains.

Root- Transverse section shows single layered epidermis, unicellular trichomes, uniseriate medullary rays. Pith is absent.[47]

3.3.7 Distribution:

E. littorale is widely distributed in South America, Africa, and Asia. It is found in all over the India up to the height 1500 feet, mostly in the coastal region.[48]

3.3.8 Chemical constituents of *Enicostemma littorale*:

Enicostemma littorale has chemical constituents like sterols, satechins, triterpenoids, volatile oil, and alkaloids. Main chemical constituents are betuline, triterpene sapogenin, and swertiamarin. It also contains erythrocentaurin reported to have α-amylase inhibitory activity. Heptacosane, nonacosane, myristic acid, stearic acid and oleic acid are also present in minor quantities.[49] Monoterpene alkaloids like enicoflavine and Gentiocrucine are also present.[50]

3.3.9 Part used **:** Whole plant

3.3.10 Dose **:** 1-3g churna, 50-100ml kwatha.[45]

3.3.11Ayurvedic pharmacology:[45]

Action on digestive system **:** Deepan, aampachan, yakritutejak, and krimighan.

Action on circulatory system **:** Rakatshodhak and shothhar.

Action on urinary system **:** Parmehaghan.

Action on skin **:** Kushatghan.

3.3.12 Reported pharmacological actions:

S. No.	Pharmacological activity	Part used	Extract	Model	Dose
1.	Anthelmintic activity[51]	Aerial part	Ethanolic extract	-	-
2.	Analgesic and anti-inflammatory activity[52]	Whole plant	Methanolic extract	Freund's adjuvant-induced arthritis	150mg/Kg
3.	Cardio protective and antihypertensive effect[53]	-	Water extract	-	1.5g/100g body wt./day
4.	Antidiabetic activity[54,55]	Whole plant	Water extract	Alloxan-induced diabetic rats.	2g/Kg
		Whole plant	Methanolic extract	Alloxan-induced diabetic rats.	2.5g/Kg/day
5.	Hepatoprotective activity[56]	Aerial part	Water extract	Paracetamol-induced hepatotoxicity in albino rats	200mg/Kg

6.	Antimalarial activity[57]	-	Methanolic extract	-	529.04 µg/ml (swertiamarin)
7.	Antiulcer activity[58]	Aerial part	Methanolic extract	Aspirin-induced gastric ulcer	200mg/Kg
8.	Antiobesity activity[59]	-	Water & ethanolic extract	High fat diet-induced obesity	200,250,400, 500mg/Kg
9.	Antihyperlipidemic activity[60]	-	Methanolic extract	Poloxamer-407-induced hyperlipidaemic model	50mg/Kg (swertiamarin)
10.	Antipyretic activity[61]	Whole plant	Ethanolic extract	-	260-780mg/Kg

Table 3.6: Pharmacological actions of mamajjaka

CHAPTER 4

4.1 SCOPE OF STUDY

Standardization is an important factor for ensuring the quality of ayurvedic dosage forms. Standardization is required to describe all measures related to the manufacturing process and quality control to develop a reproducible quality and standards for any dosage form. Ghanavati is an important formulation of ayurveda. It has been prescribed by the ayurvedic physicians since ancient time. However, sufficient data related to the standardization of ghanavati are not available. In addition to this, Mamajjakaghanavati has been used as an ethnomedicine and also by ayurvedic physicians of Gujarat mainly to treat the type-2 diabetes. It reduces the blood glucose and increases the level of serum insulin. Mamajjaka ghanavati is prepared by using mamajjaka (*Enicostemma littorale*) as the main ingredient. However, preparation of Mamajjakaghanavati is not been described by the ayurvedic scholars. So, the present study was focused on development of the standards for the preparation and evaluation of mamajjaka ghanavati.

In addition, SNEDDS of mamajjaka was also developed to improve efficacy and bio-availability.

CHAPTER 5

5.1 AIM:

Pharmaceutical development and evaluation of Mamajjaka ghanavati

5.2 OBJECTIVES:

- To authenticate the crude drug.
- To develop the method of preparation of mamajjaka ghanavati.
- To evaluate the prepared ghanavati by using analytical parameters.
- To develop SNEDDS from the Mamajjaka
- To characterized the prepared SNEDDS.
- To evaluate anti-oxidant and antidiabetic activity of ghanavati and SNEDDS by *in-vitro* models.

CHAPTER 6

MATERIALS AND RESEARCH METHODOLOGY

6.1 List of Equipment used:

S. No.	Material
1	Stainless steel containers
2	Ladle
3	Stainless steel plate
4	Cotton cloth
5	Sieves
6	Gas stove
7	Spatula
8	Beakers
9	Friability apparatus
10	Disintegration apparatus
11	Crucible
12	China dish
13	Vernier caliper
14	Microscope
15	Digital pH meter
16	Electric balance
17	Hot plate
18	Water bath
19	Hot air oven
20	Sonicator
21	UV spectrophotometer
22	Magnetic stirrer
25	Ultra centrifuge
26	Transmission electronic microscope
27	Zeta sizer

6.2 Chemical used:

S.No.	Material
1	Mamajjaka (*Enicostemma litorale* Blume)
2	Labrafil
3	Tween 80
4	Transcutol
5	DPPH
6	Acarbose
7	Chloroform
8	Hydrochloric acid
9	Ferric chloride
10	Lead acetate
11	Sodium hydroxide
12	Copper sulphate
13	Ninhydrin
14	Gelatin
15	Hager's reagent
16	Dragendroff's reagent

6.3 Research methodology:

- Identification and collection of raw drug.
- Authentication of raw drug.
- Physicochemical studies of raw drug.
- Preparation of mamajjakaghanavati.
- Evaluation of mamajjaka ghanavati.
- Preparation of SNEDDS from mamajjaka.
- Characterization of SNEDDS.
- *In-vitro* antioxidant and anti-diabetic activities of the mamajjaka ghanavati and SNEDDS.

CHAPTER 7

7. EXPERIMENTAL WORK

7.1 Collection of drug:

The whole plant of mamajjaka was collected from the Dang Forest, Gujarat, India.

7.2 Authentication of drug:

The drug was authenticated from the Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar.

7.3 Organoleptic study:

The *Enicostemma littorale* was observed for colour, odour, taste, texture etc.

7.4 Physicochemical Analysis of Mamajjaka *(Enicostemma littorale)*:

7.4.1 Foreign matter:

100 g of sample was taken and spreaded in a stainless-steel tray. The foreign matter was detected with the unaided eye. Remaining quantity of sample was weighed and percentage of foreign matter calculated.[62]

Foreign matter = (Weight of foreign matter / Weight of drug) ×100

7.4.2 Loss on drying:

5-10 gm of sample was taken (without preliminary drying) in the accurately weighed dry petri dish and kept into the dry oven at 105^0Cfor 5 hours. Then, petri dish was removed from the oven and placed into desiccator under vacuum till shelf cooling and weighed the reduced moisture content from the sample.[63]

7.4.3 Total ash:

Incinerated of the 2.5 gm of the sample into the crucible, at temperature of 450ºC for 5 hours. After shelf cooling, kept in the desiccator under vacuum. The weight of obtained ash was measured and percentage of obtained ash was calculated. [62]

Total Ash = (Weight of ash / Weight of sample) ×100

7.4.4 Acid insoluble ash:

Ash obtained from the above method was mixed with 25 ml dilute hydrochloric acid and boiled for 5 minutes. Then, mixture was filtered through ash less filter paper. The filtrate was subjected for the washing with hot water to make it chloride free and again ignited to constant weight. Percentage of acid insoluble ash was calculated after weighing obtained ash.[63]

Acid insoluble ash = (Weight of residue × Volume made) / (Weight of sample × Volume taken) × 100

7.4.5 Alcohol soluble extractive:

5gm of the sample (coarse powder) was taken in a closed conical flask with 100ml of alcohol. Conical flask was shaken frequently for 6 hours and kept undisturbed for 18 hours. Then, it was filtered by using filter paper. 25 ml of filtrate was taken in the china dish and allowed the content to evaporate. Percentage was calculated after weighing the residue.[63]

Alcohol soluble extractive value = (Weight of residue × Volume made) / (Weight of sample × Volume taken) × 100

7.4.6 Water soluble extractive:

5gms of the sample (coarse powder) was taken in a closed conical flask with 100ml of water. Conical flask was shaken frequently for 6 hours and kept undisturbed 18 hours. Then, it was filtered by using filter paper. 25 ml of filtrate was taken in the china dish and allowed the contents to evaporate. Percentage was calculated after weighing the residue.[63]

Water soluble extractive value = (Weight of residue × Volume made) / (Weight of sample × Volume taken) × 100

7.5 Qualitative analysis of *Enicostemma littorale*[64]:

7.5.1 Test for flavonoids (Shinoda test):

The extract was dissolved in methanol (50%, 1-2 ml) by heating. To an alcoholic solution of each of the extract, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

7.5.2 Test for alkaloids

7.5.2.1 Mayer's test:

One ml of aqueous extract was acidified with 2-3 drops of 1M hydrochloric acid and treated with 4-5 drops of Mayer's regent (Potassium Mercuric Iodide) Formation of a yellow or white coloured precipitates or turbidity indicate the presence of alkaloids.

7.5.2.2Dragendroff's test:

Extract was dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

7.5.3 Test for Tannin:

A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride were added to the filtrate. Formation of blackish green precipitates confirmed presence of tannins.

7.5.4 Test for Phenolic compounds:

Two to three drops of 1% ferric chloride ($FeCl_3$) solution were added in to 2 ml of 1 % extract. Deep violet colour was not produced which should be produced in the presence of phenolic compounds.

7.5.5 Test for Coumarins:

Coumarins form a yellow colour with 1% KOH in absolute ethanol. 1 ml of extract in test tubes was treated with 3-4 drops of 1% KOH in absolute ethanol.

7.5.6 Test for Reducing Sugar (Fehling's test):

To a test tube 1 ml each a Fehling's A and B solutions were added and mixed. To this 2 ml of plant extract was added and heated on a boiling water bath for 10 minutes. Formation of brick red or orange precipitate indicates the presence of reducing sugar/ carbohydrates.

7.5.7Test for Quinones:

To the test sample, sodium hydroxide is added. Formation of blue, green, or red colour indicates the presence of quinones.

7.5.8Test for Saponins (Foam Test):

0.5 g of extract was shaken with 2 ml of water. Foam produced persists for ten minutes it indicates the presence of saponins.

7.5.9Test for Proteins (Xanthoproteic Test):

The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

7.5.10Test for fixed oil and fats:

A drop of concentrated extract was pressed in between two filter papers and kept undisturbed. Oil stain on the paper indicated the presence of oils and fats.

7.6 Preparation of Mamajjak ghanavati:

Three major steps were involved in the preparation of mamajjaka ghanavati. Total six batches of mamajjaka ghanavati were prepared.

7.6.1 Preparation of Kwatha:

The authenticated crude drug was crushed to a coarse powder (sieve no. 8) and then soaked in 16^{th} part of water in stainless steel container for 15 hours. Then, it is subjected to continuous mild heat (80-90 °C) until it was reduced upto $1/8^{th}$ of its initial quantity. During the heating process, continuous stirring was done to facilitate the evaporation and avoid any deterioration due to burning of materials. After $1/8^{th}$ reduction of water, the kwatha was filtered through double folded cotton cloth and collected in separate vessel.

7.6.2 Preparation of Ghana:

The prepared kwatha was further subjected to mild heat (80-90 °C) and continuously stirred for the preparation of ghana. In the final stage, indirect heating was given to avoid denaturation of the ghana by heat.

7.6.3 Preparation of Ghanavati:

Classical method was adopted to prepare vati from Ghana. The semisolid Ghana was rolled by hands to make the round shaped vati of 500mg. Then, vati were placed in oven at temperature 45°C for 12 hours. Prepared ghanavati were subjected to various analytical parameters.

7.7 Evaluation of Mamajjaka ghanavati:

Mamajjaka ghanavati were evaluated by using different analytical parameters.

7.8 Chromatography:

7.8.1 Test solution for TLC and HPTLC

Five grams of powdered drug was taken and added 50 ml of methanol. Shaken for some time (15-20 minutes) and then mild heat is given to it for half an hour. After cooling, it is filtered. The filtrate is evaporated on water bath to approximately 10 ml and used for spotting**.**

7.8.2 Chromatographic conditions:

Solvent System	: Ethyl acetate: Methenol (8: 2)
Extract	: Methanol extract
Chamber Saturation	: 30 minute
Visualization	: Short U.V. (254 nm) and long U.V. (365nm)

7.8.3 Preparation of spray reagent (Anisaldehyde in H_2SO_4):

0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid in that order.

7.9 Preparation of SNEDDS:

7.9.1 Preparation of extract:

Soxhlet apparatus was used for the preparation of mamajjaka extract. Coarse powder of drug was used to prepare the thimble and methanol was used as solvent. The process was continued for the 24 hrs. After continuous extraction, methanol was evaporated on water bath and stored for the further use.

7.9.2 Calibration curve for extract:

0.1 ml of extract was dissolved in 100 ml of methanol. Then, 10 ml of solution was transferred into the 90 ml of methanol. Then, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of the solution were transferred to 9 ml, 8 ml, 7 ml, 6 ml, and 5 ml of methanol, respectively. After dilution, different concentrations were analyzed by using UV spectroscopy in wavelength range of 200-400 nm. The absorbance of standard solutions was measured at λ_{max} of 231 nm to prepare calibration curve.

7.9.3 Solubility studies of mamajjaka ghanavati oil in oils, surfactants, co-surfactants:

Solubility study was performed to check the solubility of extract in labrafil (oil), tween 80 (surfactant), transcutol P (co-surfactant) before formulating SNEDDS.

7.9.4 Emulsification of oils, surfactants and co-surfactants and creation of pseudoternary phase Diagram:

27 batches (B1-B27) were prepared by using various ratios of oil, surfactant and co-surfactant to prepare the SNEDDS from mammajaka ghanavati. Labrafil, tween 80 and transcutol were mixed in different ratios. The resultant mixture of each batch was subjected for the sonication on ultrasonicator for duration of 15 minutes at 37°C. Then, prepared isotropic were diluted to 200 ml by double distilled water and kept on magnetic stirrer at 500 rpm (37°C). The prepared emulsions were observed visually for their clarity and related turbidity.

7.10 Characterization of SNEDDS:

7.10.1 Entrapment Efficiency:

The proportion of encapsulated drugs was determined by centrifuging a 10 ml of SNEDDS at 15000 rpm for 60 minutes at room temperature. The supernatant was taken carefully using micropipette. Pure supernatant was then dissolved in methanol to disrupt

the vesicles and appropriate dilution was made in order to measure the content using UV spectrophotometry at 231 nm. Entrapment efficiency was calculated by the equation below[65]

% Entrapment efficiency= (Amount of encapsulated drug/ amount of total drug) × 100

7.10.2 Droplet size:

The average diameter of the SNEDDS of the mamajjaka ghanavati was measured using particle size analyzer (Beckman Coulter Desla Nano Common) in different frequencies at 25^{o}C.

7.10.3 Transmission electron microscopy (TEM):

The TEM imaging of the sample is done to observe the scanned images of the prepared SNEDDS under very high resolution 200000x to determine the particle size of the SNEDDS and entrapped drugs in the lipid.[66]

7.10.4 Zeta potential:

Zeta potential is used to identify the charge of the oil droplets of SNEDDS. The charge of the oil droplets in conventional SNEDDS is negative due to the presence of free fatty acids.[67] For the droplets in SNEDDS emulsions, a high zeta potential confirms stability and long shelf life.[68]

7.10.5 Polydespersity Index

The polydespersity index as a measure of the width of the molecular weight distribution (MWD). The MWD data analysis is presented for the correct interpretation and comparison of different, experimentally obtained, molecular weight distributions of polymers.

7.11 In vitro studies:

7.11.1 Antioxidant study

7.11.1.1 OH^- Scavenging Assay[69]

OH^- radicals were originated from $FeSO_4$ and H_2O_2 and detected by their ability to hydroxylate salicylate. 3 ml of reaction mixtures were prepared by using 1 ml of $FeSO_4$ (1.5mM), 0.7ml H_2O_2 (6mM),0.3ml sodium salicylate (20mM) and 1 ml of different dilutions of the extract. Then, these mixtures were subjected for the incubation of 1hour at 37^{o}C. After incubation, absorbance of hydroxylated salicylate complex was recorded at 562nm for different samples. Percentage of inhibition was calculated by using following formula.

$$\text{Scavenging rate} = [1-(A_1-A_2)/ A_0] \times 100$$

Where,

A_0 = absorbance of the control (without Sample),

A_1=absorbance of presence of extract and

A_2=was the absorbance without salicylate.

7.11.1.2 DPPH Assays[70]

700µl of extract was added in to the same volume of 100µM DPPH methanolic solution. Then, it was shaken vigorously and kept in the dark place for 20 min at room temperature. Lastly, absorbance was recorded at 515 nm. Percentage of inhibition was calculated by using following formula.

$$\text{Percentage inhibition (I\%)} = Abs_{control} - Abs_{Sample} / Abs_{control} \times 100$$

7.11.2 Anti-diabetic Activity

7.11.2.1 α-amylase inhibition assay[71]

Starch iodine method was used for the determination of α-Amylase activity. 10 µl of α-amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer containing different concentrations of extract. After incubation at 37 ℃for 10 min, 100 µl of the 1% starch solution was added and re-incubated for 1 hour. After re-incubation 0.1 ml of 1%

iodine solution was added and further it was diluted with 5 ml distilled water. The absorbance was taken at 565 nm.

Inhibition of enzyme activity was calculated as (%) = (A-C) × 100/ (B-C)

where, A = absorbance of the sample, B = absorbance of blank (no α-amylase), and C = absorbance of control (no starch)

CHAPTER 8

RESULTS AND DISCUSSIONS

8.1 Analysis of raw drug:

8.1.1 Organoleptic study:

S. no.	Parameters	Observation
1	Colour	Yellowish brown
2	Odour	Characteristic
3	Taste	Bitter
4	Texture	Smooth

Table 8.1: Observation of organoleptic study of mamajjaka

The organoleptic characteristics of raw material like colour, odour, taste and texture is mentioned in **Table 8.1**

8.1.2 Physicochemical study:

S. no.	Parameters (%)	Observations
1	Foreign matter	3.2
2	Loss on drying	1.377
3	Total ash	19
4	Acid insoluble ash	1.78
5	Water soluble ash	2.35
6	Alcohol soluble extractive	37.26
7	Water soluble extractive	23.04
8	pH	6.02

Table 8.2: Observation of physicochemical study of mamajjaka

Standards of the physicochemical analysis of mamajjaka were not found in Ayurvedic Pharmacopoeia of India. However, physicochemical parameters were performed during the study and results of the analysis are mentioned in **Table 8.2**

8.2 Preparation of Mamajjaka Ghanavati:

8.2.1 Ingredients of Mamajjaka Ghanavati:

Sr. No.	Dravya	Latin Name	Part Used	Quantity (gm)
01.	Mamajjaka	*Enicostemma littorale*	Whole plant	300
02.	Drinking Water (ml)	4800		

Table 8.3: Ingredients and quantity to prepare mamajjaka ghanavati

The ratio of mamajjaka and water was 1:16 for each batch of the kwatha because yield of ghana was more in comparison to 1:8 may due to the more amount of the solvent. The quantities of ingredients used for the preparation of kwatha are mentioned in **Table 8.3**.

8.2.2 Preparation of mamajjaka kwatha:

Parameter	Batches					
	I	II	III	IV	V	VI
Initial quantity of Kwatha Churna (g)	300	300	300	300	300	300
Total quantity of water (ml)	4800	4800	4800	4800	4800	4800
Total time for soaking (hrs)	15	15	15	15	15	15
Temp. during preparation of Kwatha (after 60 minutes)	90°C	90°C	90°C	90°C	90°C	90°C
Total time taken for Kwatha (minutes)	135	135	135	135	135	135
Total quantity of Kwatha obtained (ml)	610	630	620	630	615	620

Table 8.4: Observation during preparation of mamajjaka kwatha

Drug was washed and shade dried for the preparation of coarse powder and sieved through 8#. The material was soaked for 17 hrs for each batch and subjected to heat. Continuous stirring was done during the preparation of kwatha to get uniform concentration throughout the solvent and also to protect the drug from burning. The temperature was maintained below 90^{o}C throughout the process. The first batch took 135 minutes to get reduced up to 1/8th. The average yield of kwatha for all batches was approximately 621 ml. Prepared kwatha was subjected for the physico-chemical analysis. Observations of all batches of kwatha are mentioned in **Table 8.4**.

8.2.3 Evaluation of kwath:

S. no.	Parameters	Batches					
		I	II	III	IV	V	VI
1	Colour	Brown	Brown	Brown	Brown	Brown	Brown
2	Odour	Charac.	Charac.	Charac	Charac.	Charac.	Charac.
3	Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
4	pH	6	6.02	6	6	5.9	6
5	Total solid content	5.14	5.17	5.08	5.11	5.05	5.15
6	Viscosity	0.92	0.92	0.90	0.92	0.92	0.90
7	Specific gravity	0.961	0.963	0.961	0.961	0.962	0.965
8	Refractive index	0.35	0.35	0.35	0.35	0.35	0.35

Table 8.5: Analysis of mamajjaka kwath

Kwatha of each batch was evaluated by using organoleptic and physicochemical parameters. No significant difference was found in any of the parameter. Results of all batches for different parameters are mentioned in **Table 8.5**.

8.2.4 Observation during mamajjaka ghana preparation:

Parameters	Batches						Avg.
	I	II	III	IV	V	VI	
Total time taken for preparation of Ghana (min.)	200	230	210	200	220	210	211
Final quantity of Ghana obtained (g)	72.2	70.4	71.6	70.7	71.8	70.9	71.26

Table 8.6: Results obtained during preparation of mamajjaka ghana

Kwatha was further boiled for the evaporation of water to get the solid content and stirred continuously throughout the process to avoid burning of content. In final stage of ghana preparation, water bath was used to avoid the denaturation of the content by the heat. The same procedure was repeated for the all batches of the ghana. The average duration for the preparation of ghana was 211. Then, ghana was collected from the vessel and kept in an oven for 6 hrs to make it dry. Average yield of all batches was 71.26g. The observations of the preparation of ghana are mentioned in the **Table 8.6**.

8.2.5 Preparation of mamajjaka ghanavati:

Ingredients	Batches					
	I	II	III	IV	V	VI
Mamajjaka Ghana (g)	72.2	70.4	71.6	70.7	71.8	70.9
Fine powder of Mamajjaka(g)	13.85	14.18	13.96	14.14	13.92	14.10
Percentage of powder	19.18	20.14	19.49	20	19.39	19.89

Table 8.7: Quantity of ghana and mamajjaka power for preparation of ghanavati

The ghana was mixed with the fine powder of mamajjaka to roll the vati by hand. Approximately, 20 % fine powder of mamajjaka was added in each batch for the preparation of vati. Then, vati were kept for drying and stored in a well closed container for the further use. The observations of mamjjaka ghanavati are mentioned in the **Table 8.7**.

8.3 Pharmaceutical analysis of mamajjaka ghanavati:

Parameters	Batches					
	I	II	III	IV	V	VI
Colour	Blackish brown	Blackish brown	Blackish brown	Blackish brown	Blackish brown	Blackish brown
Odour	Charac.	Charac.	Charac.	Charac.	Charac.	Charac.
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Shape	Round	Round	Round	Round	Round	Round
pH	5.6	5.7	5.6	5.5	5.7	5.5
Weight variation	Pass	Pass	Pass	Pass	Pass	Pass
LOD at 105 ^{o}C (%)	7.09	7.00	7.41	7.27	11.08	11.02
Total ash (%)	11.02	10.89	11.18	11.07	10.94	11.11
Acid insoluble ash (%)	0.22	0.27	0.30	0.32	0.28	0.18

Water soluble extract (%)	86.21	84.00	85.06	84.23	86.39	85.09
Alcohol soluble Extract (%)	12.96	13.02	12.87	12.89	13.05	12.95
Disintegration test (min)	15:07	15:00	15:05	15:07	15:04	15:01
Friability (%)	0.098	0.097	0.098	0.098	0.097	0.099
Diameter (cm)	0.9	0.9	0.9	0.9	0.9	0.9
Hardness (kg/cm$^{2)}$	5.34	5.35	5.35	5.35	5.34	5.35

Table 8.8: Observation during pharmaceutical analysis of mamajjaka ghanavati

No significant variation was observed in the organoleptic and physico-chemical parameters in different batches of ghanavati. The average disintegration time was recorded 15.04 min. the percentage of friability was very low without significant difference in the different batches. The average percentage of friability percentage was 0.097. The average diameter of vati in all batches was 0.9 cm. Weight variation in each batch is below 5% which is acceptable for the vati of 500 mg. Whereas, in hardness test 5.34 (kg/cm^2)The results of organoleptic and physico-chemical parameters of different batches of vati were mentioned in the **Table 8.8**

8.4 Qualitative test for mamajjaka ghanavati:

S. No.	Components	Chemical tests	Observation	Results
1	Phenolic compound	_	Brown	_
2	Tannins	Ferric chloride test	Greenish black colour	+
3	Flavonoids	Shinoda test	Yellow colour	+
4	Coumarins	KOH	Yellow colour	+
5	Steroidal glycosides	L. Burchard's test	Reddish brown ring	+
6	Alkaloids	Mayer's test	Red Precipitates	+
		Dragendroff's test	Red precipitates	
7	Protein	Xanthoprotein test	Yellow colour	+
8	Quinones	Sodium hydroxide test	Green colour	+
9	Anthraquinone glycoside	Borntrager test	Greenish brown	_
10	Saponins	Foam test	Formation of foam	+
11	Reducing sugars	Fehling's test	Brick red colour	+
12	Fixed oil and Fats	Spot test	Oil stain on paper	+

Table 8.9: Observation during phytochemical screening of mamajjaka ghanavati

Qualitative analysis of vati revealed the presence of tannins, flavonoids, coumarins, steroidal glycosides, alkaloids, protein, quinones, saponins, reducing sugars, fat and fixed oil. The results of qualitative tests are mentioned in the **Table 8.9**

8.5 Chromatographic analysis:

8.5.1 TLC analysis of mamajjaka ghanavati

Sample	Solvent system	Rf. value (short UV)	Rf. value (long UV)	Rf. value (After spraying with analseldehyde)
Mamajjaka ghanavati	Ethyl acetate : Methenol (8: 2)	0.08,0.91	0.08,0.91	0.08,0.71,0.91

Table 8.10: Rf. values of mamajjaka ghanavati

Two Rf were recorded in the TLC of mamajjaka ghanavati under the short wavelength. Whereas, one additional Rf was observed after spray. The results are mentioned in **Table 8.10**

8.5.1.1 Image of TLC plate run on Ethyl acetate: Methenol (8: 2):

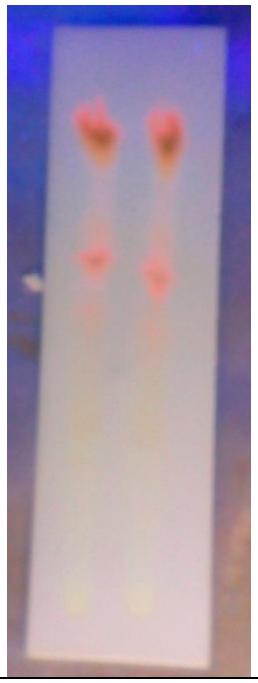


Figure:8.1 TLC in visual light

Figure:8.2 TLC in Short UV

8.5.2 HPTLC analysis of mamajjaka ghanavati:

8.5.2.1 HPTLC of mamajjaka ghanavati run on Ethyl acetate: Methenol (8: 2):

S. No.	Rf. values in HPTLC	Rf. value in TLC
1	0.03	
2	0.08	0.08
3	0.14	
4	0.24	
5	0.30	
6	0.47	
7	0.57	
8	0.81	
9	0.91	0.91

Table 8.11: HPTLC showing Rf. values of mamajjaka ghanavati

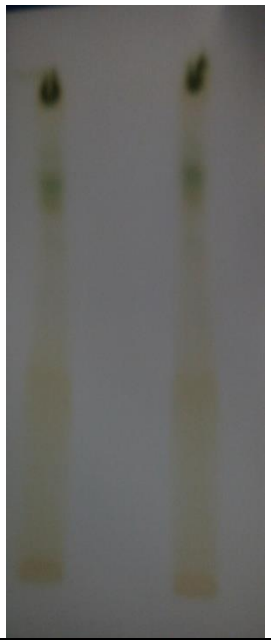


Figure: 8.3 HPTLC plate

8.6 SNEDDS:

8.6.1 Extraction of mamajjaka:

Sr. No.	Observations	Batch					Average
		I	II	III	IV	V	
1	**Weight of drug (g)**	25	25	25	25	25	25
2	**Volume of solvent (ml)**	150	150	150	150	150	150
3	**Yield of extract (%)**	10.90	10.23	11.02	10.88	10.78	10.79
4	**Duration (hours)**	24	24	24	24	24	24

Table 8.12: Observation of extraction process

Total 5 batches were prepared for the extraction of mamajjaka. The quantity of drug and methanol were taken for the extraction of each batch was 25 g and 150 ml respectively. The duration of process was 24 hrs which provided average 10.79% yield of methanolic extract.

8.6.2 Calibration of curve:

S. No.	Conc. (µg/ml)	Absorption
1	0	0
2	1	0.124
3	10	0.238
4	20	0.418
5	30	0.619
6	40	0.823

Table 8.13: Calibration curve of mamajjaka ghanavati

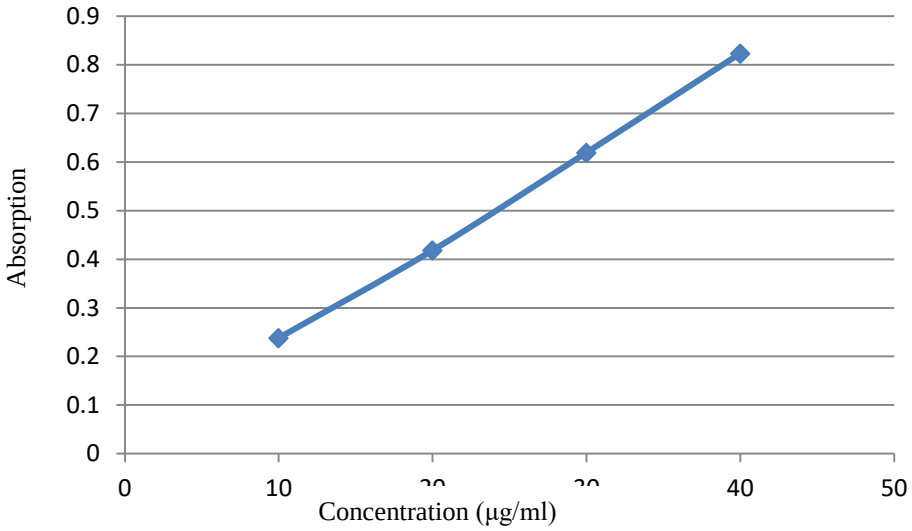


Figure:8.4 Standard calibration curve

The extract concentration 1mg/ml was scanned on 200-400nm to determine the λmax, shown in the Figure 4 to determine the linearity to use the λmax 231nm to see the absorbance of the serial dilution solution of the extract. Absorbance was found respectively in the different concentration and plotted the standard graph using Conc. Vs Abs. and found the linear equation. Y=0.019x+0.047, R^2 =0.988, it is closed to the 1 and confirm the linearity of the lambert law.

8.6.3 Emulsification of oils, surfactants and co-surfactants and creation of pseudoternary phase Diagram:

Batch no.	Oil	Surfactant	Co-surfactant	Sample	Water	Result
1	0.1(10%)	0.45(45%)	0.45(45%)	150µl	200ml	Creaming
2	0.2(20%)	0.40(40%)	0.40(40%)	150µl	200ml	Very turbid
3	0.3(30%)	0.35(35%)	0.35(35%)	150µl	200ml	Creaming
4	0.4(40%)	0.30(30%)	0.30(30%)	150µl	200ml	Turbid and creaming
5	0.5(50%)	0.25(25%)	0.25(25%)	150µl	200ml	Creaming
6	0.6(60%)	0.2(20%)	0.2(20%)	150µl	200ml	Creaming
7	0.7(70%)	0.15(15%)	0.15(15%)	150µl	200ml	Creaming
8	0.8(80%)	0.10(10%)	0.10(10%)	150µl	200ml	Creaming
9	0.9(90%)	0.05(5%)	0.05(5%)	150µl	200ml	Turbid and creaming
10	0.1(10%)	0.3(30%)	0.6(60%)	150µl	200ml	Cracking
11	0.2(20%)	0.27(27%)	0.53(53%)	150µl	200ml	Cracking
12	0.3(30%)	0.23(23%)	0.47(47%)	150µl	200ml	Cracking
13	0.4(40%)	0.2(20%)	0.40(40%)	150µl	200ml	Cracking
14	0.5(50%)	0.17(17%)	0.33(33%)	150µl	200ml	Creaming
15	0.6(60%)	0.13(13%)	0.27(27%)	150µl	200ml	Cracking
16	0.7(70%)	0.1(10%)	0.2(20%)	150µl	200ml	Cracking
17	0.8(80%)	0.07(7%)	0.13(13%)	150µl	200ml	Cracking
18	0.9(90%)	0.03(3%)	0.07(7%)	150µl	200ml	Creaming
19	0.1(10%)	0.6(60%)	0.3(30%)	150µl	200ml	Clear
20	0.2(20%)	0.53(53%)	0.27(27%)	150µl	200ml	Cracking
21	0.3(30%)	0.47(47%)	0.23(23%)	150µl	200ml	Cracking
22	0.4(40%)	0.40(40%)	0.2(20%)	150µl	200ml	Cracking
23	0.5(50%)	0.33(33%)	0.17(17%)	150µl	200ml	Cracking
24	0.6(60%)	0.27(27%)	0.13(13%)	150µl	200ml	Cracking
25	0.7(70%)	0.2(20%)	0.1(10%)	150µl	200ml	Cracking

26	0.8(80%)	0.13(13%)	0.07(7%)	150µl	200ml	Creaming
27	0.9(90%)	0.07(7%)	0.03(3%)	150µl	200ml	Creaming

Table 8.14: Results of different ratio of oil, surfactant and co-surfactant

27 batches were prepared by using various ratios of oil, surfactant and co-surfactant to prepare the SNEDDS from mamajjaka ghanavati. The prepared emulsions were checked visually for their clarity and related turbidity. B19 was found clear in all the batches of the SNEDDS. Whereas, B2, B4, and B9 were turbid, creaming were observed in B1, B3, B5, B6, B7, B8, B14, B18, B26, and B27. Phase separation was occurred in B10, B11, B12, B15, B17, B20, B21, B22, B23, B24, and B25. On the basis of above observations a ternary phase diagram was drawn to find out the SNEDDS region. The detail observations of different batches are mentioned in **Table 8.14.**

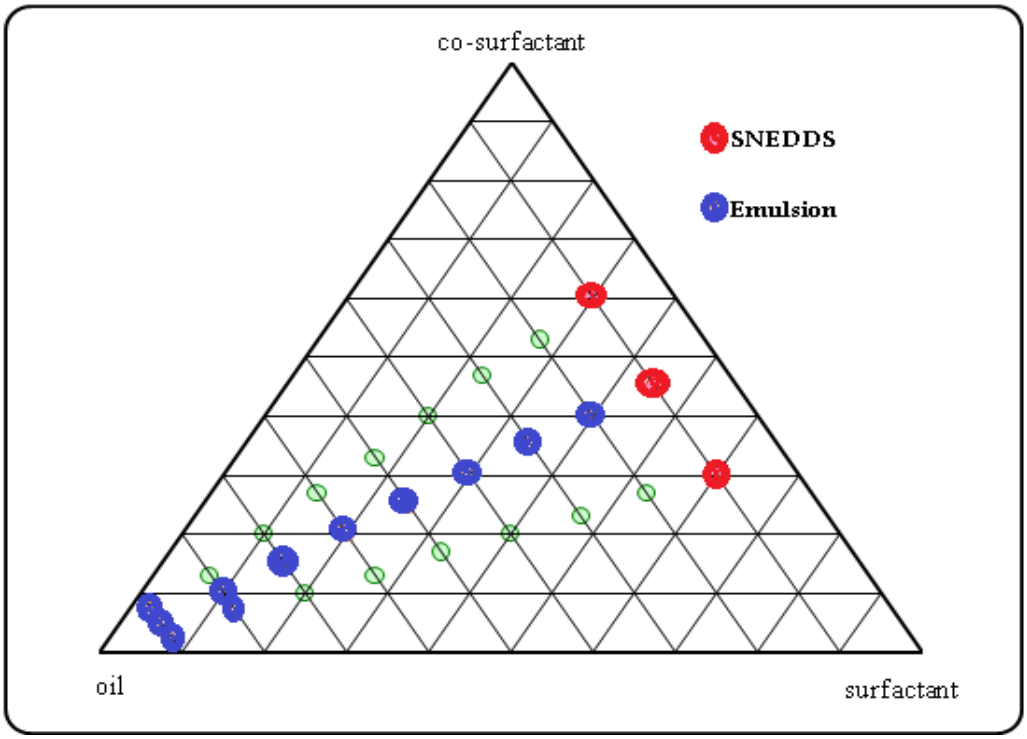


Figure: 8.5 Ternary phase diagram showing SNEDDS, Emulsion and Phase

8.6.4Characterization of optimized batch of SNEDDS:

8.6.4.1 % Drug loading

The % drug loading capacity of the optimized batch of SNEDDS was found 92%.

8.6.4.2 Droplet size analysis

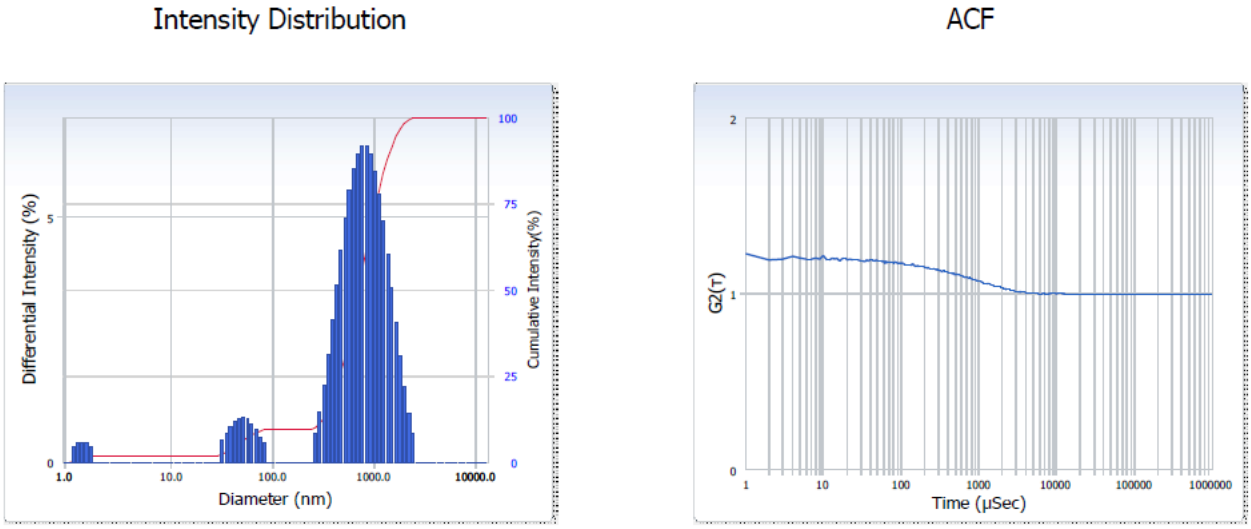


Figure:8.6 Droplet size analysis of optimizes batch of SNEDDS of mamajjaka ghanavati

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1.5	0.2
2	55.3	14.7
3	902.7	439.6
4	0.0	0.0
5	0.0	0.0
Average	820.8	488.3
Residual :	2.930e-003	(O.K)

Cumulants Results

Diameter	(d)	: 545.8	(nm)
Polydispersity Index	(P.I.)	: 0.377	
Diffusion Const.	(D)	: 9.037e-009	(cm^2/sec)
Molecular Weight		: 1.225e+010	
Measurement Condition			
Temperature		: 25.1	(°C)
Diluent Name		: WATER	
Refractive Index		: 1.3328	
Viscosity		: 0.8858	(cP)
Scattering Intensity		: 28253	(cps)
Attenuator 1		: 10.12	(%)

The mean droplet size of optimized batch of SNEDDS was 545.8 nm. Whereas, polydispersity index of SNEDDS was 0.377.

8.6.4.3 Zeta potential

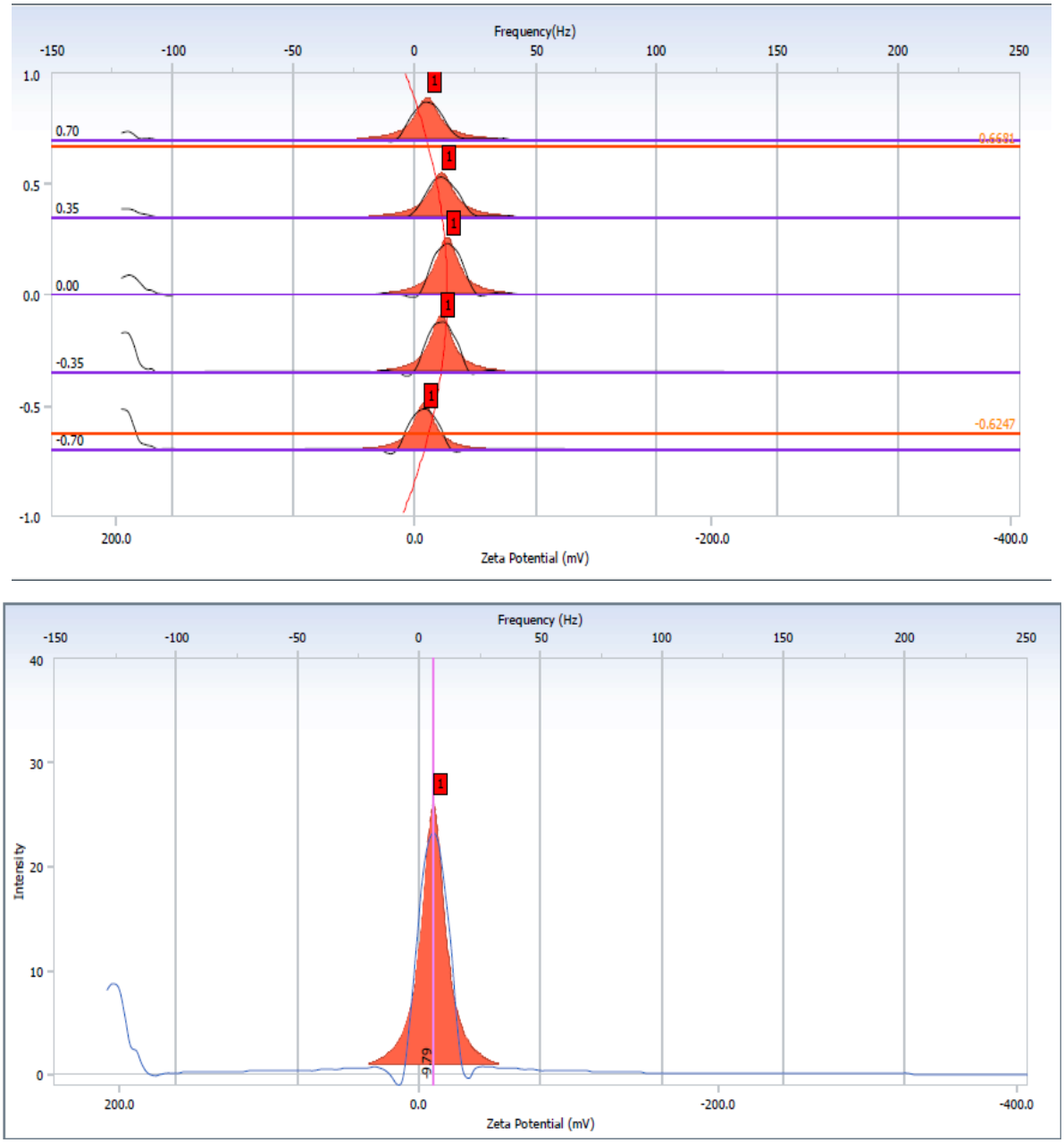


Figure:8.7 Zeta potential of the optimized batch of SNEDDS of mamajjaka ghanavati

Zeta Potential	: -22.08	(mV)	Doppler shift	: 13.49	(Hz)
Mobility	: -1.713e-004	(cm²/Vs)	Base Frequency	: 120.8	(Hz)
Conductivity	: 0.2680	(mS/cm)	Conversion Equation	: Smoluchowski	

Zeta Potential of Cell			Diluent Properties		
Upper Surface	: 17.05	(mV)	Diluent Name	: WATER	
Lower Surface	: 55.45	(mV)	Temperature	: 24.7	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8939	(cP)
Avg. Electric Field	: -16.30	(V/cm)	Dielectric Constant	: 78.4	
Avg. Current	: -0.22	(mA)			

Zeta potential of SNEDDS of mamajjaka ghanavati was found to be -22.8mV which was attributed to the steric stabilization of SNEDDS due to the presence of surfactant and co-surfactant system.

8.6.4.4 Transmission Electron microscopy(TEM):

The Transmission electron microscopic scanning of the prepared SNEDDS were done at the SAIF lab under the premisses of the Punjab University, Chandigarh. The following scanned images were obtained.

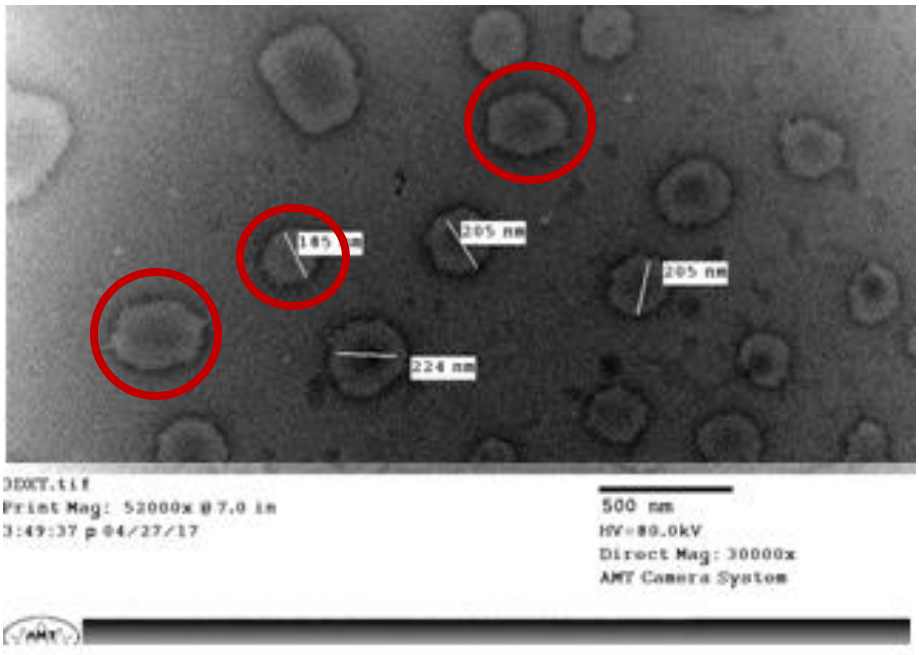


Figure:8.8

TEM image of SNEDDS loaded with the mamajjaka ghanavati

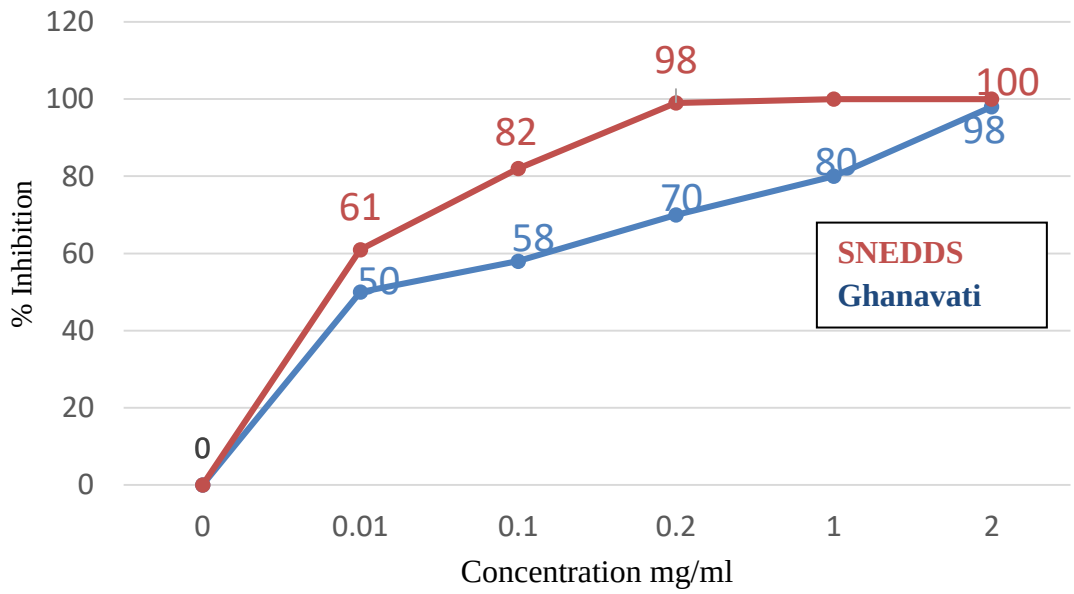
The TEM image clearly indicated spherical droplets of mamajjaka ghanavati loaded SNEDDS in nanometer range. The image confirmed the particle size and shape of SNEDDS that they were in spheres and less than 500 nm in size.

8.7 In-vitro study on mamajjaka ghanavati and SNEDDS:

8.7.1 OH^- scavenging activity:

S.No.	Conc. (mg/ml)	% inhibition of ghanavati	% inhibition of SNEDDS
1	0.01	50	61
2	0.1	58	82
3	0.2	70	99
4	1	80	100
5	2	98	100

Table 8.15: % inhibition of mamajjaka ghanavati and its SNEDDS

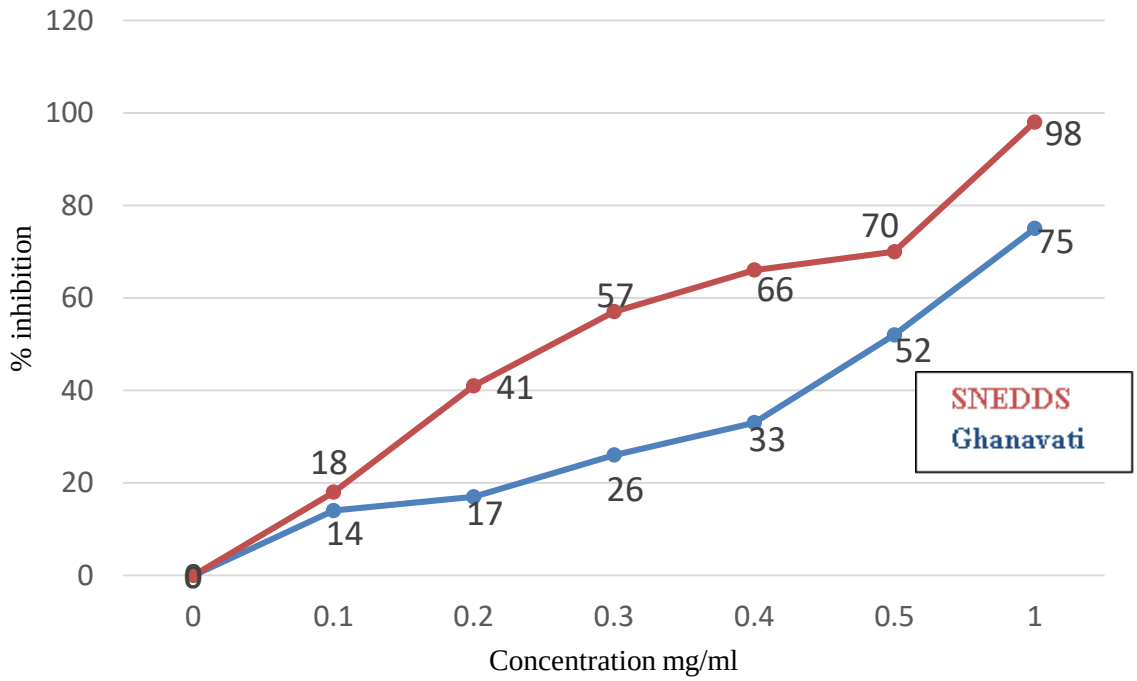


Different concentrations of mamajjaka ghanavati and SNEDDS were used to check its anti-oxidant activity by OH^- scavenging assay. % inhibition of SNEDDS were comparatively high than mamajjaka ghanavati. The IC_{50} value of SNEDDS was -0.34 and IC_{50} value of mamajjaka ghanavati was 0.25.

8.7.2 DPPH assay:

Sr. No.	Conc. (mg/ml)	% inhibition of extract	% inhibition of formulation
1	0.1	14	18
2	0.2	17	41
3	0.3	26	57
4	0.4	33	66
5	0.5	52	70
6	1	75	98

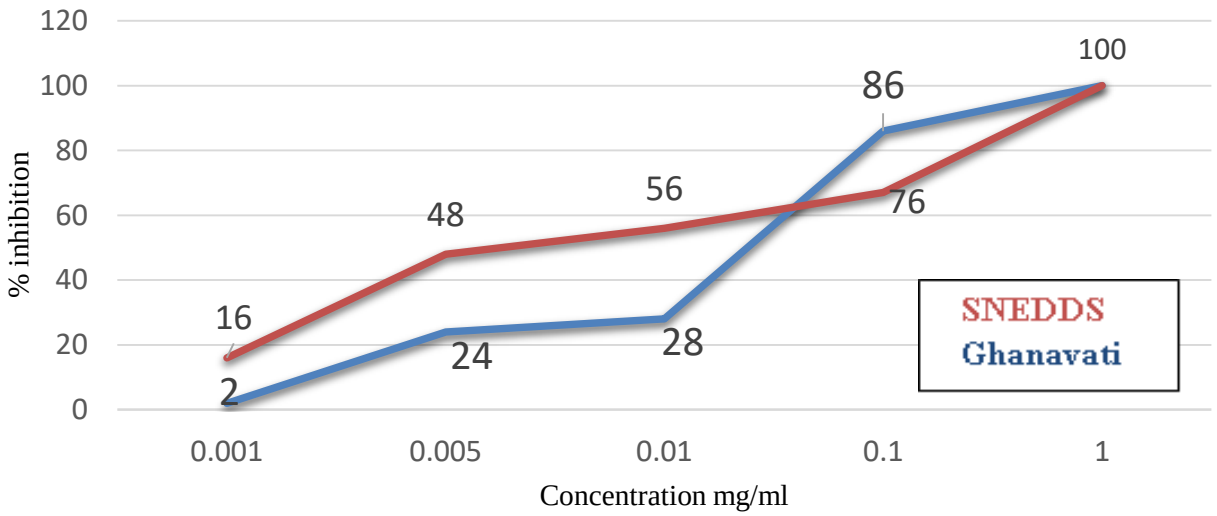
Table 8.16: % inhibition of mamajjaka ghanavati and its SNEDDS (OH^- scavenging assay)



8.7.3 α-amylase activity:

S. No.	Conc. (mg/mi)	% inhibition Extract	% inhibition Formulation
1	0.001	2	16
2	0.005	24	48
3	0.01	28	56
4	.1	86	67
5	1	100	100

Table 8.17: % inhibition of mamajjaka ghanavati and its SNEDDS



To evaluate and compare the anti-diabetic activity α-amylase assay was performed. SNEDDS was having high % inhibition as compare to mamajjaka. IC_{50} of SNEDDS was 0.093 whereas IC_{50} of mamajjaka ghanavati was 0.26.

CHAPTER 9

CONCLUSION AND FUTURE SCOPE

For 300 gm of *Mamajjaka Kwatha Churna* (sieve size no. 08), 16 times water (4800 ml) should be taken and reduced upto $1/8^{th}$ (600 ml) to prepare *Kwatha* at 90^{o}C temperature. Again this *Kwatha* should be heated for the preparation of Ghana. The average yield of ghana was 71.26 g (Average 23.75%). Vati prepared by traditional method complying with the all standards. Labrafil, tween-80 and Transcutol P were selected as oil, surfactant, and co-surfactant respectively. The optimized design suggested that use of 0.1 ml of Labrafil M 1944CS, 0.6 ml of tween 80 and 0.3 ml of Transcutol P could give SNEDDS with 500 nm mean droplet size, 92 % drug loading and -22 mV zeta potential. *In-vitro* antioxidant and anti diabetic activities of mammajaka ghanavati and SNEDDS revealed that nano formulation is the better dosage form for the mammajaka than ghanavati.

Future scope: *In-vivo* studies are required to compare the anti-diabetic effect of mamajjaka gahavati and SNEDDS.

CHAPTER 10

References

1 Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, et al. et al. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Plants Res. 2010;4(12):1089–1101.

2 Upadhyay UM, Goyal RK. Efficacy of *E. littorale* in type 2 diabetic patients. Phyto Res. 2004;18:233–235.

3 Praveena P, Sudarsanam D. In vitro antimicrobial activity studies on Enicostemma littorale (Lam), Raynal Whole plants. Int J Curr Res. 2011;11(3):123–124.

4 Mishra S, Shukla P. *In vitro* anthelmintic activity of *Enicostemma littorale* Blume. Int J Pharma Sci Res. 2011;2(5):1193–1196.

5 Jaishree V, Badami S, Kumar MP, Tamizhmani T. Antinociceptive activity of Swertaimarin isolated from *Enicostemma axillare*. Phytomedicine. 2009;16: 227–232.

6 Mukundray NB, Chauhan K, Gupta S, Pillai P, Pandya C, Jyoti V, et al. et al. Protective effect of *Enicostemma littorale* Blume methanolic extract on Gentamicin induced Nephrotoxicity in rats. Am J Inf Dis. 2011;7(3):83–90.

7 Roy SP, Niranjan CM, Jyothi TM, Shankrayya MM, Vishawanath KM, Prabhu K, et al. et al. Antiulcer and anti-inflammatory activity of aerial parts of *Enicostemma littorale* Blume. J Young Pharm. 2010;2(4):369–373.

8 Kavimani S, Manisenthilkumar KT. Effect of methanolic extract of *Enicostemma littorale* on Dalton's aseptic lymphoma. J Ethnopharmacol. 2000;71:349–352.

9 Vaijanathappa J, Badami S, Bhojraj S. *In vitro* antioxidant activity of *Enicostemma axillare*. J Heal Sci. 2008:524–528.

10 Prince PSM, Srinivasan M. *Enicostemma littorale* Blume aqueous extract improves the antioxidant status in alloxan induced diabetic rat tissues. Acta Pol Pharm Drug Res. 2005;62(5):363–367.

11 http://pib.nic.in/newsite/PrintRelease.aspx?relid=136482 (03.04.2017)

12 Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by

Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 269.

13 Sushruta, Sushruta Samhita, Uttaratantra, edited with Ayurveda TatvaSandipika Hindi commentary by Ambikadutta Shastri,13th edition, Chaukhamba Sanskrit Sansthan, Varanasi, 2002, 24/20

14 Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 445.

15 Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 451.

16 Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007;452-453.

17 Vagbhata, Astanga Hridaya, Sutrasthana, Vidyotini Bhashatika by Kaviraj Atridev Gupta, 14th edition, Chaukhamba Sanskrit Sansthan, Varanasi, 2003, 27/41.

18 Bhavamishra, Bhavaprakasha, Part 2, Edited with Hindi commentary by Pandit Bhramashankar Misra, 11th edition, Chaukhamba Sanskrit Bhavan, Varanasi, 2007, 2/1

19 Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, utara khanda. Chaukhamba surbharti prakashan Varanasi; 437.

20 Vaidhya Yadavji Trikamaji, Siddha Yoga Sangraha, 11th Edition, Shri Baidhnath Ayurved Bhavan; 4.

21 Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, Mdhyam khanda. Chaukhamba surbharti prakashan Varanasi; 210.

22 Dhundi S N. Pharmaceutical standardization of Guduchi Ghana. International research journal of pharmacy 2011; 2(11):104.

23 Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 446.

24 Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, Mdhyam

khanda. Chaukhamba surbharti prakashan Varanasi; 195.

25 The Ayurvedic Pharmacopoeia of India. Part II (Formulations) Volume-1. First edition; 97.

Panda P, Meher SK. Tablet & tableting in ayurveda (vati kalpana)- A review. IAMJ 2016; 07:1219.

27 The Ayurvedic Pharmacopoeia of India. Part II (Formulations) Volume-1. First edition; 146

28 Savale K Sagar. A Review- Self Nanoemulsifying Drug Delivery System (SNEDDS). International Journal of Research in Pharmaceutical and Nano Sciences. 4(6), 2015, 385-386.

29 Sandeep Kumar Singh, Priya Ranjan Prasad Verma and Balkishen Razdan. Development and characterization of a lovastatin loaded selfmicroemulsifying drug delivery system, Pharmaceutical Development and Technology, 15(5), 2010, 469-483.

30 Rajalakshmi R, Mahesh K, Ashok Kumar C K. A Critical Review on Nano Emulsions, International Journal of Innovative Drug Discovery, 1(1), 2011, 1-8.

31 Gursoy RN, Benita S, self emulsifying drug delivery systems (SNEDDS) for improved oral delivery for lipophilic drugs. Biomed Pharmacother 2004; 58: 173-182.

32 Gershanik T, Benita S. Self -dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur J Pharm Sci 2000; 50:179-188.

33 Pouton CW. Self emulsifying drug delivery system: accessment of efficiency of emulsification. Int J Pharm 1985; 27: 335- 338.

35 Bajaj H, Bisht, S, Yadav M, SinghV, Singh M.Self emulsifying drugdelivery system: an approach to enhance Bioavailability. Int J Pharm 2011 Research, 3, 59-79.

36 Patel PA, Chaulang GM, Akolkotkar A, Mutha, SS, Hardikar SR, Bhosale AV.Self Emulsifying Drug Delivery System: A Review. Research J Pham Tech 2008; 1: 313-23.91

37 Shukla Jill B, Koli Akshay R, Ranch Ketan M and Parikh Rajesh K, Self-Micro Emulsifying Drug Delivery System, Pharma Science Monitor, An International Journal of Pharmaceutical Sciences, 1(2), 2010, 13-33.

38 K, Pawar YB, Bansal AK. Self emulsifying drug delivery systems: A strategy to improve

oral bioavailability. Curr Res Info Pharm. 2010; 11: 42-49.

39 Khan AW, Kotta S, Ansari SH, Sharma RK, Ali J. Potentials and challenges in self-nanoemulsifying drug delivery systems. Expert Opinion on Drug Delivery 2012;9:1305-17.

40 Taha E, Ghorab D, Zaghloul A-a. Bioavailability assessment of vitamin A self-nanoemulsified drug delivery systems in rats: a comparative study. Medical Principles and Practice 2007;16:355-9.

40 Shukla A. A review of a potential hypoglycemic plant (Enicostemma littorale blume). European journal of biomedical and pharmaceutical sciences 2014. Vol. 2; 1:154-155. Rajamani S, Trumalai T; Pharmacognosy of Enicostemma littorale: A review. Asian pacific journal of tropical biomedicine. 2013; 3(1): 79

42 Srivastava S, Kumar V. Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. Inforesights publishing 2012; 5.

43 Vaibhav A, Singh O P. A concise review on “mamajjaka” (Enicostemma littorale). Asian Journal of science and technology 2016. Vol-07; 07: 3204.

44 Sharma PV. Dravyaguna Vijnana. Vol. 2. Chaukhambha Bharti Academy Varanasi 2012; 704-705.

45 Maroo J, Vihas T. Glucose lowering effect of aqueous extract of Enicostemma littorale Blume in diabetes: a possible mechanism of action. Journal of Ethnopharmacology 2002; 317.

46 Sanmugarajah V, Thabrew I. Phyto, physicochemical standardization of medicinal plant Enicostemma littorale, Blume. IOSR Journal of Pharmacy 2013. Vol. 4; 2: 52

47 The Ayurvedic Pharmacopoeia of India. Part-I. Volume- VI.First edition;115.

48 Rathod M C, Dhale D A, Pharmacognostic characterization of Enicostemma littorale Blume. Int. J. Res. Ayurveda Pharma 2013; 4(6): 893.

49 Hassan N, Ahamad J. Rapid preparative isolation of erythrocentaurin from Enicostemma littorale by medium-presssure liquid chromatigraohy, its estimation by high-pressure thin layer chromatography, and its α-amylase inhibitory activity. J. Sep. Sci. 2015; 1-7.

50 Dhanavathy G. Immunohistochemistry, histopathology, and biomarker studies of

swertiamarin, a secoiridoid glycoside, prevents and protects streptozotocin-induced B-cell damage in Wistar rat pancreas. Italian Society of Endocrinology 2015; 2.

51 Mishra S, Mishra A. In-vitro anthelmintic activity of Enicostemma littorale Blume. International journal pharmaceutical sciences and research 2011; Vol. 2(5) : 1194-1195.

52 Shabi M M. Uthrapathy S. Analgesic and anti-arthritic effect of Enicostemma littorale Blume. Advances in Bioscience and Biotechnology 2014; 1018-1020.

53 Bhatt N, Chavda M. Cardioprotective and antihypertensive effects of Enicostemma littorale Blume extract in fructose-fed rats. Can. J. Physiol. Pharmacol 2012; 1065-1070.

54 Srinivasan M, Padmanabhan M. Effect of aqueous Enicostemma littorale Blume extract on key carbohydrate metabolic enzymes, lipid peroxides and antioxidants in alloxan-induced diabetic rats. Journal of pharmacy and pharmacology 2005; 57: 497-500.

55 Maroo J, Ghosh A. Antidiabetic Efficacy of Enicostemma littorale methanol extract in alloxan-induced diabetic rats 2003. Pharmaceutical biology. Vol. 41; 5: 388-390.

56 Gite V N, Pokharkar R D. Hepato-protective activity of Enicostemma axillare in paracetamol induced hepato-toxicity in albino rats. International journal of pharmacy & life sciences 2010; 50-53.

57 Soni S, Gupta S. In vitro anti plasmodial activity of Enicostemma littorale. American journal of infectious Diseases 2009; 5(3): 259-260.

58 Roy S P, Niranjan C M. Antiulcer and anti-inflammatory activity of aerial parts Enicostemma littorale Blume. Journal of young pharmacists 2010; 2(4): 369-370.

59 Garg A, Singh R. Antiobesity activity of aqueous and ethanol extracts of Enicostemma littorale in high fat diet induced obese rats. International journal of phytomedicine 2014; 6: 433-440.

60 Vaidya H, Rajani M. Antihyperlipidaemic activity of swertiamarin, a secoiridoid glycoside in poloxamer- 407 - induced hyperlipidaemic rats. The Japanese Society of Pharmacognosy and Springer 2009; 437.

61 Okokon J E, Nwafor P A. Antipyretic and antimicrobial activities of crude lesf of Enicostemma Littorale. Asian Pacific Journal of Tropical disease 2012; 2(6):442-444.

62 The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed.1999, part I, Vol.-II,Apx-2 Page no-190.

63 The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed.1999, part I, Vol.-II,Apx-2 Page no191.

64 T. Prashant, Bimlesh Kumar et.al. Phytochemical screening and Extraction : A Review,International Pharmaceutical Science 2011;1: 98-106

65 Pushpendra Kumar Jain, Navneet Khurana et.al.,Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system, J Liposome Res,2013, 1532-2394

66 Felix Borrnet..Thought about next generation (S)TEM instrument in science, micrin 2015; 90: 1-5

67 Gershanik T, Benita S. Positively charged self-emulsifying oil formulation for improving oral bioavailability of progesterone. Pharmaceutical Development And Technology 1996;1:147-57.

68 Gupta S, Kesarla R, Omri A. Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. ISRN pharmaceutics, 2013.

69 Hua Wang,Xiang Dong Gao et.al, In vitro and in Vivo antioxidant activity of aqueous extract from choerospondias axillaries fruit, Food Chemistry 106 (2008) 888-895

70 Monica Locatelli,Roberto gindro et.al.,Study of the DPPH-Scavenging activity: Development of a free softwear for the correct interpretation of data,Food chemistry,114 (2009),889-897

71 Sudha P, Smita S Zinjarde et.al., Potent a-amylase inhibitory activity of Indian Ayurvedic medicinal plants, BMC Complementary and Alternative Medicine 2011, 11:5

CHAPTER 11

Appendixes

INDIA'S LARGEST UNIVERSITY

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education, Transforming India

TOPIC APPROVAL PERFORMA

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : P570-NN7::M.Pharm. (Ayurveda)

COURSE CODE : APH623 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** PHRRGD0037

Supervisor Name : Dr. Manish Vyas **UID :** 17410 **Designation :** Associate Professor

Qualification : _____ **Research Experience :** _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Shivangni Raj	11501454	2015	Y1553	8679368425

SPECIALIZATION AREA : Ayurvedic Pharmacy **Supervisor Signature:** _____

PROPOSED TOPIC : Pharmaceutical development and evaluation of Mamajjaka ghanvati

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	6.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	7.00
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	7.00
5	Social Applicability: Project work intends to solve a practical problem.	7.00
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	8.00

PAC Committee Members		
PAC Member 1 Name: Dr. Amit Mittal	UID: 13145	Recommended (Y/N): NA
PAC Member 2 Name: Saurabh Singh	UID: 12208	Recommended (Y/N): Yes
PAC Member 3 Name: Dr. S. Tamilvanan	UID: 16391	Recommended (Y/N): NA
PAC Member 4 Name: Dr. Navneet Khurana	UID: 18252	Recommended (Y/N): NA
DAA Nominee Name: Dr. Sazal Patyar	UID: 17050	Recommended (Y/N): NA

Final Topic Approved by PAC: **Pharmaceutical development and evaluation of Mamajjaka ghanvati**

Overall Remarks: Approved

PAC CHAIRPERSON Name: 11045::Dr. Monica Gulati **Approval Date:** 29 Nov 2016

ਬੋਟੈਨੀਕਲ ਐਂਡ ਐਨਵਾਇਰਨਮੈਂਟਲ ਸਾਇੰਸਿਜ਼ ਵਿਭਾਗ
ਗੁਰੂ ਨਾਨਕ ਦੇਵ ਯੂਨੀਵਰਸਿਟੀ, ਅੰਮ੍ਰਿਤਸਰ - 143 005

Department of Botanical & Environmental Sciences
Guru Nanak Dev University, Amritsar - 143 005, India

(Established by the State Legislature Act No. 21 of 1969)
Accredited at "A" grade level by NAAC and awarded "University with Potential for Excellence" status by UGC

Ref. No. 1338 Bot. & Env. Sc.
Dated 24-11-2016

To Whom It May Concern

The plant specimen(s) brought by Ms. Shivangi Raj
Regn No. 1T501454 student of M.Pharmacy (Ayurveda) L.P.U, Phagwara (Pb.)
belongs to the following species.

1. Mamajjka
2.
3.

Signature of Student Shangai

Herbarium Assistant

Teachers Incharge

Head
Deptt. of Botanical & Environmental Sciences
Guru Nanak Dev University
Amritsar-143005.