

Pharmaceutical Development and Evaluation of Oral Dosage form Prepared from Spinach

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF PHARMACY (AYURVEDA)

In

Rasashastra & Bhaishajya Kalpana

By

Anil Kumar Sah

11105847

Under the guidance of

Dr. Manish Vyas

Associate Professor

Rasashastra & Bhaishajya Kalpana



School of Ayurvedic Pharmaceutical Sciences

Lovely Professional University

Punjab 144411

November

2017

Statement by the Candidate

This is to submit that this written submission in my project report entitled “**Pharmaceutical development and evaluation of oral dosage form prepared from spinach**” 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 will be cause 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. Manish Vyas
Associate Professor

Anil Kumar Sah
Reg. No: 11105847

Certificate by Supervisor

The work described in this project report entitled “**Pharmaceutical development and evaluation of oral dosage form prepared from spinach**” has been carried out by **Anil Kumar Sah (11105847)** under my supervision. I certify that this is his 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 oral dosage form prepared from spinach**” has been carried out by **Anil Kumar Sah** 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 support during 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** COD Lovely School of Ayurvedic Pharmaceutical Sciences, Lovely Professional University for giving his support to proceed my project work.

I am very grateful to **Prof. & 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. Ramchandra Sah** and **Mrs. Punam Sah** 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 **Shivangni Raj, Saveena Chauhan, Arun Kumar, Swati Sharma, Sweta pathyarach, Ambika Thakur, Preeti Kalsi, Neha Bhatia, Balaji Panigrahi, and Anuj Kumar** 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 to lead me towards achieving my goals.

INDEX

Sn.no	Content	Page No.
1	Introduction	1-2
2	Terminology	3
3	Literature Review	4-10
3.1	Literature Review of Spinach	4-8
3.1.1	Taxonomical Classification	4
3.1.2	Vernacular Names	4
3.1.3	Geographical Source	4
3.1.4	Cultivation/Harvest and Storage of Spinach	4
3.1.5	Botanical Description	5
3.1.6	Morphological Character of Spinach	5
3.1.7	Ayurvedic Literature of Spinach	6-7
3.1.8	Reported Pharmacology Activity	7-8
3.2	Review of Phytosome	8-10
3.2.1	History of Phytosome	8-9
3.2.2	Advantage of the Phytosome	9
3.2.3	Commercial Nutraceutical Phytosome Products	9-10
3.2.4	Marketed Formulation of Phytosome	10
4	Rationale and scope of the study	11
5	Aim and Objective	12
6	Material and research methodology	13
6.1	Instruments	13
6.2	Research Methodology	13
7	Experimental Work	14-25
7.1	Procurement and Authantication	14
7.2	Drug Analysis	14
7.2.1	Physicochemical Analysis of Spinach	14-15
7.2.2	Qualitative Analysis of Spinach	15-16
7.2.3	Nutritional value Analysis	17-18

7.3	Preparation of spinach extract	19
7.4	Characterization of the extract	20-21
7.4.1	T.L.C. Analysis	20
7.4.2	HPTLC Analysis	20-21
7.4.3	UV. Spectrophotometer Analysis	21
7.4.4	IR Spectrophotometer Analysis	21
7.5	Preparation of Phytosome	22
7.6	Characterization of Phytosome	23-24
7.7	<i>In-vitro</i> study of the Phytosome loaded with Spinach	24-25
7.7.1	Antioxidant Activity	24
7.7.2	Anti-Diabetic Activity	24-25
8	Result and Discussion	26-39
9	Conclusion	40
10	References	41-46
11	Appendixes	47-

LIST OF TABLES

Table No.	Name of Tables	Page No.
3.1	Ayurvedic literature of the Spinach.	6-7
3.1.8	Research based on the pharmacological activities of Spinach	7-8
3.2.3	Marketed phytosome nutraceuticals	9-10
3.2.4	Marketed Phytosome	10
8.1	Organoleptic character of the spinach	26
8.2	Physicochemical Analysis of Spinach	26
8.3	Qualitative Analysis of spinach	27
8.4	Nutritional value Analysis	27
8.5	Extraction of the Spinach	28
8.6	Thin Layer Chromatography	28
8.10	Different batches for the preparation of the phytosome loaded with lipid or drugs	32
8.14.1.a	Antioxidant Activity (OH ⁻)	37
8.14.1b	Antioxidant Activity(DPPH)	38
8.14.2.	Anti-diabetic Activity	39

ABBREVIATIONS TABLE

USD	-	United State Dollar
CAGR	-	Compound Annual Growth Rate
Mg	-	Milligram
Kg	-	Kilogram
Cm	-	Centimeter
Mm	-	Millimeter
TBA	-	Thiobarbituric acid
FOX	-	Ferrous Oxidation-xylene Orange
ABTS	-	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic Acid)
TPC	-	Total Phenolic Content
ORAC	-	Oxygen Radical Absorbance Capacity
CCA	-	Circulating Cathodic Antigen
Ref	-	References
No	-	Number
Sr.	-	Serial
T.L.C	-	Thin Layer Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
IR	-	Infrared spectroscopy
UV	-	Ultraviolet spectrophotometer
MWD	-	Molecular Weight Distribution
PDI	-	Polydispersity index
TEM	-	Transmission Electron Microscopy
DPPH	-	2, 2-diphenyl-1-picrylhydrazyl
Abs.	-	Absorbance
Rf.	-	Retention Factor

ABSTRACT

Background: Nutraceuticals are the functional food that promote the health and manage the disease. The Indian pharmaceutical industry is rapidly growing with the growth rate of 19.5 % per year in the field of nutraceuticals. The global market of nutraceuticals was 142.1 billion USD in 2011 and is expected to reach up to 204.8 billion USD in 2017. The increasing demand of nutraceuticals leads to the scope of research and drug development. Moreover, Ayurveda has great potential to provide established and time-tested drugs to develop effective nutraceuticals. Spinach is described by the Charaka and Shushruta in shaka varga and also a rich source of carbohydrate, proteins, amino acids, fats, minerals, metals including the natural steroids. So, present study was designed to develop phytosome by using spinach. **Objective:** Development, characterization and evaluation of antioxidant and anti-diabetic activities of phytosome prepared from spinach. **Result and Discussion:** The phytosome were prepared by using solvent evaporation method and characterized by entrapment efficiency (84%), particle size (188.3 nm), polydisperse index (0.357), and zeta potential (-41.4 mV). Further, phytosome was evaluated and found effective in *in-vitro* models of anti-diabetic and antioxidant activity. **Conclusion:** The study revealed that phytosome of spinach prepared and also has effective anti-diabetic and antioxidant activity.

Keywords: - Nutraceutical, Ayurved, Disease, Phytosome

CHAPTER I

INTRODUCTION

'Ayurveda' is a science of life and longevity.¹ Aims of Ayurveda include the maintenance of the health of the healthy individual and treatment of diseases.² Ayurveda has three modes of treatment i.e. Hetu (cause), Ling (symptom) and Aushad (medicine).³ Aushad is incorporated in all branches of Ayurveda as a mode of treatment including Rasayana.^{4,5} Rasayana is the branch of Ayurveda which deals with the nourishment of the body and tissues to enhance memory, intelligence, luster, complexion, voice and reduce the effect of aging.⁶ Acharya Charak,⁷ Acharya Sushruta,⁸ and Acharya Dalhan have described the different types of Rasayana. Ajasrika is a type of Rasayana described by the Acharya Dalhan deals with a daily intake of proper diet (Aahar) specifically milk and ghee.⁹ Proper diet is the best source of complete nutrition which provides strength, complexion, and vitality.¹⁰ Drugs and their formulations have also been prescribed by the different scholars of Ayurveda if Aahar is not able to deliver the required amount nutrition. These drugs and formulations can be correlated with the nutraceuticals. Because they are also used to provide the complete nutrition to the body.

Nutraceuticals and dietary supplement are the functional food that promote the health and manage the disease.¹¹ These are utilized from decades for the development of the body. Nutraceuticals have an advantage due to their natural sources and polyherbal combinations.¹² The Indian pharmaceutical industry is rapidly growing, in the field of nutraceuticals with the growth rate of 19.5% per year.¹³ The global market of nutraceuticals was 142.1 billion USD in 2011 and is expected to reach up to 204.8 billion USD in 2017. The Transparency Market Research, Albany, New York has reported growth in the market of nutraceuticals at a CGrowth capital of 6.3%.¹⁴ The increasing demand leads to the new scope of research and drug development in the field of nutraceuticals. Besides, Ayurveda has great potential to provide established and time-tested drugs to develop potent nutraceuticals. Some examples of such drugs are Liquorice, Ginseng, Onion, Ginger, Spinach, Aloe and Turmeric etc.¹⁵

The research on nutraceuticals not only limited up to the exploration of a better drug molecule; it is also focused on the better dosage forms or drug deliveries to overcome the limitations of conventional dosage forms including powder, granules, syrup, and tablets etc. Conventional dosage forms are having problems related to the solubility, dose, bioavailability, safety, efficacy,

and stability.¹⁶ Nano-delivery systems have several benefits over the conventional dosage forms including the broader applications for nutraceuticals, small dose of active molecule, protection of active molecule against the oxidation and other effects, better efficiency for antioxidants and preservatives, better bioavailability of drugs, stable composition of drug molecule, and better stability of drug and its carrier.

CHAPTER II

TERMINOLOGY

Extraction	Extraction is a technique for separate a desire substance using the polar solvents.
Novel Drug Delivery system	The new technique for the delivery of drug into the body for having its maximum efficacy
Phytosome	Phytosome contains two words i.e. Phyto (plant derivative) and Some (vesicles). The drug is entrapped into the lipid bilayer
Characterization	The art of describing the character.
Anti-diabetic	Which inhibit the excessive glucose
Antioxidant	The substance that inhibits the oxidation

CHAPTER III

REVIEW AND LITERATURE

3.1. Literature review of *Spinacia oleracea*.

3.1.1 Taxonomical Classification¹⁷

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Subdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Caryophyllidae
Order	:	Caryophyllales
Family	:	<i>Chenopodiaceae/Amranthaceae</i>
Genus	:	<i>Spinacia</i>
Species	:	<i>oleracea</i> L.

3.1.2 Vernacular Names¹⁸

English	:	Spinach
Hindi	:	Pinni/Palak
French	:	Epinard
German	:	Spinat
Italian	:	Spinacio

3.1.3 Geographical Source

The nutritional and mediational benefits of the leafy vegetables like spinach provides to better support of the human wellbeing. It is mainly found in the central and western Asia and limited to a specific geographical location in India and all over the world.¹⁹

3.1.4 Cultivation/Harvest and Storage of Spinach

Spinach can be cultivated in early spring or winter season. Although, it can also be grown throughout the year under the mild temperature about 21⁰C. It can be propagated by the seedling under the wide range of soil having fertility and well-drained soil. Seedling seeds are 0.5 inches deep and covered with light soil. Harvest of the spinach not done until it's

leaves reach desired size it will be ready to harvest in about 4-5 weeks. It can be harvested at once and cut at the base of the plants.²⁰

3.1.5 Botanical Description

Spinach (*Spinacia oleracea*) is a perennial leafy vegetable grown throughout the world. It belongs to *Amaranthaceae* family.

Leaves:- The colour of leaves is yellowish green to dark green. Leaves are simple, ovate to triangular moreover flat or curved. The arrangement of the leaves is alternate. The size of leaves varies in length between 2-30 cm and in width between 1-15cm.

Flower:- Flower is small yellow to green in colour, the diameter is generally between 3-4 cm, growing plants contains small, hard, dry, and lumpy fruit clusters. These clusters contain several seeds of 5-10 mm size.^{21,22}

Stem:- It is erect from 30 to 60cm long, round, smooth, hollow stem have more than normally thickened and fleshy, usually to retain water (succulent), some reddish.²³



Fig 3.1. a) Leaf, b) Stem, c) Root

3.1.6 Macroscopical Characters of the Leaves:

Spinach is green leafy herbaceous vegetables. It's cultivated in the cold season in different regions of the world. Spinach leaf is green, simple, ovate to triangulate flat or slightly

wrinkled or curled in shape. Leaves are alternate and different variables size from 2-30cm long and 1-15cm broad.



Fig 3.2 Leaf

3.1.7 Classical description of Spinach

Table No. 3.1: Ayurvedic literature of the Spinach.

S.No	Classical Texts	Description of spinach	Properties	Ref.
1	Charak Samhita	The Palak is leafy vegetable describes under the Shak varga.	Rasa -Madhur Guna -Guru,Ruksha Veerya -Seeta Vipaka -Madhur Prabhav -Malbhedhaka	24
2	Sushruta Samhita	Palak has similar properties like chaulai leafy vegetable.	Rasa -Madhur Guna -Ruksha Veerya -Seeta Vipak -Madhur Prabhav -Vatajanaj,	25

			Balabardhak, madanashak, etc.	
3	Astangsanghardya	Palak is mentioned in a shak varga	Rasa- Madhur Guan- Guru, Pistchil Veerya- Seeta Vipak- Madhur Prabhav- Sukravardhak	26
4	Shankar Niganthu	Palak is described in shak varga	Rasa- Madhur Guna- Guru, Pistchil Veerya- Seeta Vipak- Madhur Prabhav- Kaphakarak, Pittasamaka, Visaharak.	27
5	Bhavprakash Niganthu	Palak is described in the shak varga. It is found in all over the world. It has specific character and properties. It is used in different diseases like ashmari, soath, aanavikar etc.		28

3.1.8 Reported Pharmacological Activity

Table No. 3.1.8 Research based on the pharmacological activities of Spinach

Sr. No	Extract of Spinach	Pharmacological Activity	In vivo Models	In Vitro Models	Dose	Ref. No
1	Methanoilc Extract	Protection against Gamma Radiation	Adult male Swiss mice	-	1100mg/kg/day	29
2	Aqueous Extract	Antioxidant Activity	-	TBA and FOX Assays	-	30
3	Spinach Phytoconstituents	Antioxidant Activity	-	ABST Assays	-	31
4	Water and Ethanolic Extract	Antioxidant Activity	-	TPC, ORAC, and CAA Assays	50mg/ml	32
5	Critical fluid Extract	Antioxidant Activity	-	RAS and DPPH scavenging Assays		33
6	Glycolipids	Inhibition of Mammalian DNA Polymerases	-	NUGC-3 human gastric		34

				human cancer cell line Assays		
7	Alcoholic Extract	Hepatoprotective Activity	CCl ₄ treated Rat models	-	100-200mg /kg /day for 7 days	35
8	Homogenated Extract	Clastogenic activity	7-12 week old male NMRI mice		150mg /kg	36
9	Glycolipid fraction	Antitumor Activity	Female BALB/c mice mouse colon-26 cell lines		100 µg/mL	37
10	Ethanollic extract	Antitumor Activity		MTT Assays models cell line		38
11	Aqueous Extract	CNS Depressant effect	The locomotors activity in the rat model		150 -200 mg/kg	39
12	Fresh juice extract and Methanolic Extract	Anthelmintic Activity	Earthworms model Assays		20mg/ml	40

3.2. Review of Phytosome

Lipid compatible molecular complex over the water soluble components is called as Phytosome.⁴¹ Phytosome contains two words i.e. Phyto (plant derivative) and some (cell-like). Phytosome is prepared by the special process by treating plant extract or phytoconstituents with phospholipids to produce lipid complex which enhances the absorption and bioavailability of phytoconstituents.⁴²

3.2.1 History of Phytosome

Phytosome was developed in 1989 by the Italian scientist Indena S.p.A and markedly observing the strong bonding affinity of polyphenols with the phospholipids in their intact plant tissue. The team of the scientist was working on the formulations of polyphenol to

enhance the bioavailability for its oral administration. Conversion of polyphenols into the phytosome led to significant increase in their bioavailability. The polyphenol was chemically reacted with a phospholipid to prepare phytosome. The prepared phytosome was compared with polyphenol for their bioavailability and efficacy.⁴³

3.2.2 Advantage of the Phytosomes

1. Phytosome enhances bioavailability due to their complex with phospholipid and also improves the absorptions.
2. The absorption of non-lipophilic phytoconstituents in the intestinal lumen can be increased by the formulated Phytosome.
3. The better absorption of active constituents decreases their therapeutic dose
4. Phytosome is widely used in cosmetic preparation due to the better penetration of the skin.
5. Phytosomes has better stability.
6. Phosphatidycholine is not only the carrier; it is also has better hepatoprotective activity and nutritional value.⁴⁷

3.2.3 Commercial Nutraceutical Phytosome Products

Table No.3.2.3: Marketed phytosome nutraceuticals

Sr.No.	Natural Sources	Phytoconstituents complex	Phytosomal Products	Dose (mg)	Ref.No
1	<i>Panax ginseng</i>	Ginsenoside	Ginseng Phytosome	150	44
2	<i>Cammellia sinensis</i>	Epigallocatechin, catechin, epicatechin-3-O-gallate	Green Tea Phytosome	400	17
3	<i>Vitis vinifera</i>	Resveratrol, Catechin, Quercitin	Biovin and leucoselect phytosome, Masquiller`s Phytosome	50-100	17,45
4	<i>Crateegus oxyacanthoides</i>	Hyperin, quercitin	Hawthron Phytosom	100	17
5	<i>Echniacea angustifolia</i>	Echinacosides and high molecular weight polysaccharide (Inulin)	Echniacea Phytosome	400	46

6	<i>Glycine max</i>	Genistein and daidzein	Soyselect Phytosome		17
---	--------------------	------------------------	---------------------	--	----

3.2.4: Marketed Formulation of Phytosome^{47,48}

Table No. 3.2.4. Marketed Phytosome

Sr.No.	Marketed Phytosome	Source	Dose (mg)
1	Silybin phytosome	<i>Silybum maricanum</i>	120
2	Ginkgo Phytosome	<i>Ginko biloba</i>	120
3	Ginseng phytosome	<i>Panax ginseng</i>	150
4	Green Tea Phytosome	<i>Thea sinesis</i>	50-100
5	Grape Phytosome	<i>Vitis vinifera</i>	50-100
6	Hawthorn Phytosome	<i>Crataegus species</i>	100
7	Echinacea Phytosome	<i>Echinacea angustifolia</i>	400
8	Curcumin Phytosome	<i>Curcuma longa</i>	200-300

CHAPTER IV

RATIONALE AND SCOPE OF THE STUDY

The increasing demand of nutraceutical leads the new scope of research and development in the field of functional foods and nutraceuticals. Ayurveda has a great potential to provide the established and effective drugs or their combinations as nutraceuticals. These drugs and combinations are the rich sources of all the nutrients required to develop a nutraceuticals. Spinach is one of such drug which contains amino acids, vitamins, calories, carbohydrates, fats & fatty acids, micro & macronutrients, and sterols etc.⁴⁹ Phytosome is lipid compatible molecular complex over the water soluble components is called as Phytosome. Phytosome contains two words i.e. Phyto (plant derivative) and some (cell-like). It enhances bioavailability due to their complex with phospholipid and also improves the absorptions. Hence, it was selected as a carier of the drug for the development of nutraceutical.

CHAPTER V

Aim

- Pharmaceutical development and evaluation of oral dosage form prepared from spinach.

Objectives of the Study

- Development of the phytosome from the spinach.
- Characterization of the prepared phytosome.
- *In-vitro* evaluation of the phytosome.

CHAPTER VI

MATERIAL AND RESEARCH METHODOLOGY

6.1 Instruments

- Rota evaporator
- UV. Spectrophotometer
- IR. Spectrophotometer
- HPTLC
- Particle Size Analyzer
- Zeta Potential
- TEM

6.2 Research Methodology

- Procurement of the spinach
- Authentication of the spinach
- Physicochemical evaluation of the spinach
- Development of the phytosome prepared by spinach
- Characterization of the phytosome
- *In-vitro* anti-oxidant activity of phytosome
- *In-vitro* anti-diabetic activity of phytosome.

CHAPTER VII

EXPERIMENTAL WORK

7.1 Procurement and Authentication

7.1.1 Procurement of herbal drug:

The drug was procured from the local market of Phagwara.

7.1.2 Authentication of the Drug:

The drug was authenticated from the Gurunanak Dev University, Amritsar as per the ref.no.1334.

7.2 Drug Analysis

7.2.1 Physicochemical Analysis of Spinach

7.2.1.1 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.⁵⁰

7.2.1.2 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°C for 5 hrs. 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.⁵¹

7.2.1.3 Total ash

Incinerated of the 2.5 gm. of the sample into the crucible, at temperature of 450°C 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.⁵⁰

$$\text{Total Ash} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

7.2.1.4 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.⁵⁰

$$\text{Acid insoluble Ash} = \frac{\text{Weight of residue} \times \text{volume made}}{\text{Weight of sample} \times \text{volume taken}} \times 100$$

7.2.1.5 Alcohol soluble extractive

5 gm 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.⁵¹

$$\text{Alcohol-soluble extractive} = \frac{\text{Weight of residue} \times \text{volume made}}{\text{Weight of sample} \times \text{volume taken}} \times 100$$

7.2.1.6 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.⁵¹

$$\text{Water-soluble extractive} = \frac{\text{Weight of residue} \times \text{volume made}}{\text{Weight of sample} \times \text{volume taken}} \times 100$$

7.2.2. Qualitative Analysis of spinach⁵²

7.2.2.1 Tests for alkaloids

Mayer's test: 1 ml of aqueous extract was acidified with 2-3 drops of 1M HCl and treated with 4-5 drops of Mayer's reagent (Potassium Mercuric Iodide). No colour or precipitate or turbidity was observed.

Dragendroff's test: Extract was dissolved in dilute HCl and filtered. It was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). No colour or precipitates or turbidity was observed.

Wagner's reagent: Extract was dissolved in dilute HCl and filtered. Filtrate was treated with Wagner's reagents (Iodine potassium iodide solution). No colour or precipitates or turbidity was observed.

Hager's reagent: Extract was dissolved in dilute HCl and filtered. Filtrate was treated with Hager's reagents (saturated solution of picric acid). No colour or precipitates or turbidity was observed.

7.2.2.2 Tests for saponin glycoside

Foam test: 0.5 g of extract was shaken with 2 ml of water. Foam was produced and persisted for ten minutes.

7.2.2.3 Test of reducing sugar

Benedict's test: Extract and Benedict's reagent was mixed in equal quantity and kept in boiling water for 5 minutes. Then, green colour was observed.

Fehling's test: 1 ml each a Fehling's A and B solutions were added in 2 ml of extract and kept in boiling water for 10 minutes. Brick red precipitates was observed.

7.2.2.4 Test for monosaccharides

Barfoed's test: Mixed equal volume of barfoed's reagent and extract. Then, it was kept in boiling water for 1-2 min. After cooling, red precipitates were observed.

7.2.2.5 Test for Proteins (Xanthoproteic Test):

The extract was treated with few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins.

7.2.2.6 Test for amino acid:

Ninhydrin test: 3 ml of extract was heated with 3 drops of 5% Ninhydrin solution for 10 min. Formation of purple color indicated the presence of amino acid.

7.2.2.7 Test for Steroids

Salkowski reaction: 2 ml extract, 2 ml chloroform and 2 ml con. Sulphuric acid were mixed and shaken well. Red colour appeared in chloroform layer and fluorescent greenish yellow was observed in the acid layer.

7.2.3 Nutritional value Analysis

The sample were analyzed for the presence of fat, ash, proteins, crude fiber, vitamins and carbohydrate.⁵³

7.2.3.1 Determination of Crude Protein

100 mg homogenized plant material with 3ml of 10% trichloroacetic acid was centrifuged at 10000 rpm for 1hr and supernatant was discarded. Sediment was treated with 3 ml of 1 N NaOH and subjected for heating for 7 min in water bath. After shelf-cooling, solution was centrifuged again for 5-10 min at 5000 rpm. Then, 5 ml reagent containing 100 parts of 2% solution of Na₂CO₃ and 1 part of 2% of sodium potassium tartrate was added and allowed to stand for 10-15 min. Again, Folin and Ciocalteu's phenol reagent was added and allowed to stand for 30min for development of colour. Absorbance was measured at 700 nm after the development of the colour.⁵⁴

7.2.3.2 Determination of Fat/Oil

Accurately weighed air dried plant material was taken and extracted with petroleum ether at temperature 40-60°C by using the soxhlet apparatus. Extract was dried in a desiccator and solvent was removed under vacuum at 40°C. Then, percentage was calculated after weighing the residue.⁵⁵

7.2.3.3 Determination of Carbohydrate

Percentage of carbohydrate was calculated by determining the percentage of moisture, crude fiber, ash, crude fat and crude protein.⁵⁶

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ Crude fiber} + \% \text{ Ash} + \% \text{ Crude Fat} + \% \text{ Crude Protein})$$

7.2.3.4 Determination of Vitamin A

0.5 gm. leaf extract was homogenized and saponified with alcoholic KOH on a water bath for 30 min. Then, extract was shifted to a separating funnel and mixed well with 10-15 ml of petroleum ether. Lower aqueous layer was removed and upper layer of petroleum ether containing carotenoid was collected. The process was repeated for complete removal of aqueous layer. The final volume of the petroleum ether extract was measured and its absorbance was recorded by using UV spectroscopy at 450 nm.⁵⁷

7.2.3.5 Determination of Vitamin C

1gm of grounded sample of spinach was taken in conical flask. Then, 10 ml of 0.05 M oxalic acid and 0.02 M EDTA solution were added in to the conical flask and allowed to stand for 24 hrs. Then, solution was filtered by using Whatman's filter paper No.1. 2.5 ml of filtrate was transferred to 25 ml brown volumetric flask. Then, 2.5 ml of oxalic acid, 2.5 ml of EDTA solution, 0.5ml of metaphosphoric acid with acetic acid, 1 ml of 5% H₂SO₄ solution and 2 ml of ammonium molybdate solution were added subsequently in the volumetric flask. Then, 25ml with distilled water was used to make up the volume. Lastly, absorbance was recorded at 760 nm by UV spectrometer.⁵⁸

7.3 Preparation of spinach extract

The fresh leafy spinach is procured from the local market and washed with the tap water. Then, it was subjected for the drying to remove the water and following steps were followed to prepare the extract of spinach.⁵⁹

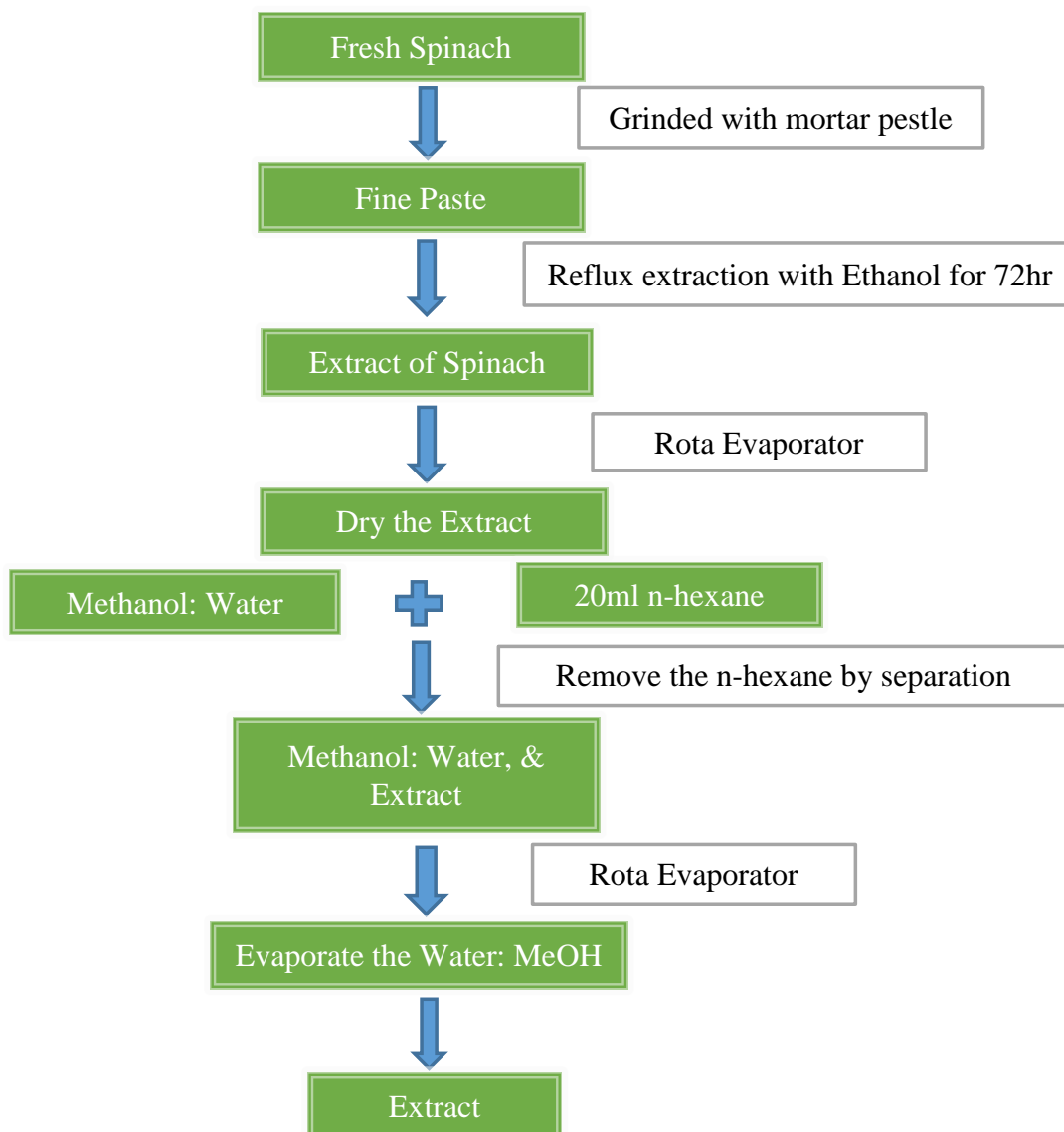


Fig: 7.1.6 Flow chart of the extraction of spinach

7.4 Characterization of the extract

7.4.1 Thin Layer Chromatography (T.L.C.) Analysis

TLC (Thin Layer Chromatography) is an important or easy technique for the qualitative and quantitative analysis of the compound or herbal drugs. It consists two phases one is stationary phase and another is mobile phase. In the analysis of spinach, TLC was developed using suitable solvent system.⁶⁰

7.4.1.1 Preparation of sample

10mg of spinach extract was taken and added 10 ml of the methanol. Shaken well until the extract was dissolved in the methanol and filtered it with the filter paper. Concentrate the filtrate on water bath and stored in the closed container until used for spotting.

7.4.1.2 Chromatographic conditions:

Solvent System	: Chloroform: Isopropyl Alcohol: Acetic Acid (12:8:1)
Extract	: Methanol extract
Chamber Saturation	: 30 minutes
Visualization	: Short U.V. (254 nm) and long U.V. (365nm)

7.4.2 High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC is the advanced form of TLC used for the qualitative and quantitative analysis by enhancing the separation and resolution of the compounds. Fine particle size of stationary phase ensures the better efficiency of the separation and resolution in HPTLC.⁶¹ The prepared sample of the extract and chromatographic conditions were same as mentioned above.

7.4.2.1 Chromatographic conditions:

Application mode	: CAMAG TLC Scanner "Scanner_180710" S/N 180710
Plate Size	: 6 x 10
Plates	: Precoated silica gel GF ₂₅₄ plates
Chamber saturation	: 30 min
Development time	: 30 min
Scanner	: CAMAG TLC Scanner
Scanning mode	: Linear at 254 nm
Detection	: D2 &W

Data system	: win CATS Planar Chromatography Manager
Drying device	: Oven
U.V. Spectrum	: 200 nm to 700 nm

7.4.3 UV. Spectrophotometer Analysis.

UV spectrophotometer was used to check the absorption of the extract to find out the actual concentration of the drug in a solvent by using calibration curve method.

7.4.3.1 Preparation of stock solution

The 1gm extract of spinach is dissolved in 100 ml of double distilled water to prepare the 10 mg/ml of stock solution.

7.4.3.2 Dilution of the Sample

Different concentrations including 1, 10, 20, 30, 40 mg/ml of stock solution were prepared by serial dilution and absorbance of different concentration were recorded at 230 nm. Calibration curve was prepared by using absorbance of different concentrations.

7.4.4 IR Spectrophotometer Analysis

Infrared spectroscopy analyzes the interaction between infrared light and molecule. It can be analyzed by measuring the absorption, emission and reflection. This technique generally to determine the functional groups of organic and inorganic molecules.

7.5 Preparation of Phytosome

7.5.1 Schematic diagram of Phytosome prepared by Spinach^{62,63}

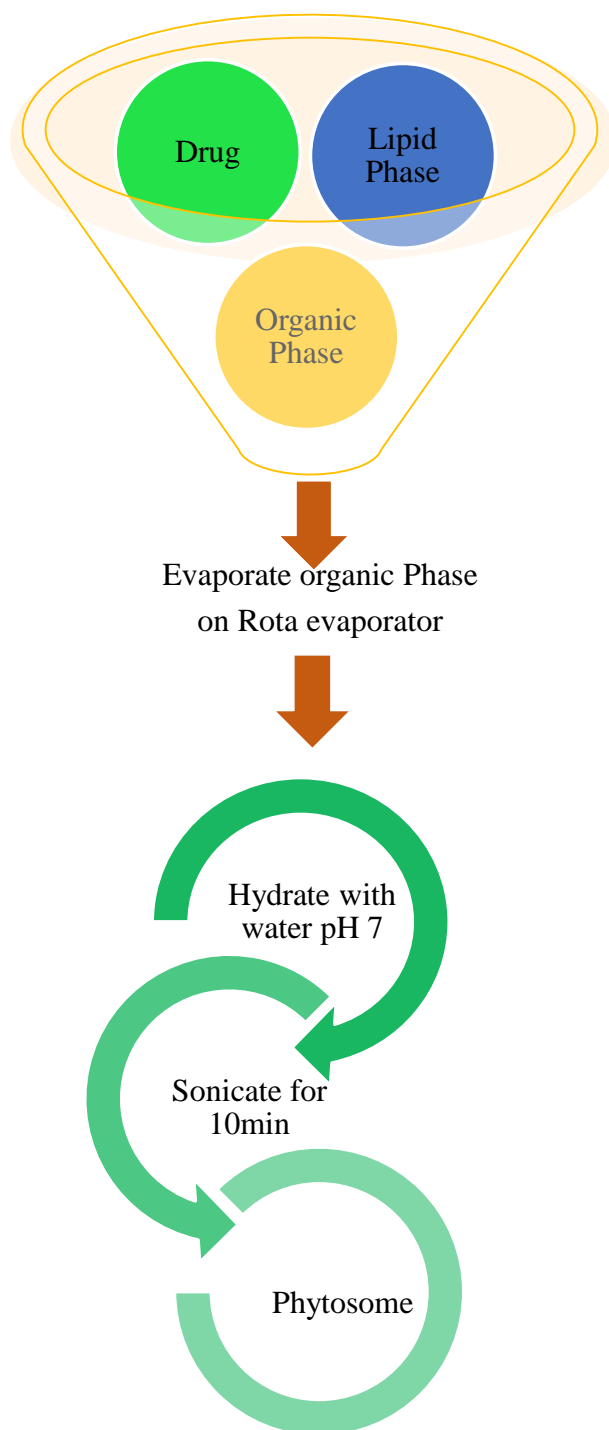


Fig 7.2.1 Schematic diagram of preparation of Phytosome

7.6 Characterization of Phytosome

7.6.1 Entrapment Efficiency

The proportion of encapsulated drugs was determined by centrifuging a 10 ml of phytosome at 15000 rpm for 60 minutes at room temperature. The supernatant was taken carefully using micropipette. Pure supernatant was then dissolved in the methanol to disrupt the vesicles and appropriate dilution was made in order to measure the silymarin content using UV spectrophotometry at 288 nm. Percentage of entrapment efficiency and loading capacity were determined by the following equation.⁶⁴

$$\% \text{ Entrapment Efficiency} = \frac{\text{Amount of encapsulated drug}}{\text{Amount of total drug}} \times 100$$

$$\% \text{ Loading Capacity} = \frac{\text{Amount of encapsulated drug}}{\text{Total Lipid}} \times 100$$

7.6.2 Particle Size Analysis

The average diameter of the phytosome of the spinach lipid complex was measured using particle size analyzer (Beckman Coulter) in different frequencies at 25°C.

7.6.3 Zeta potential

Zeta potential is an electro-kinetic parameter indirectly determined by the surface charge of the particles when suspended in the polar media having the charge negative or positive. It is measured by Beckman coulter.^{65,66,67}

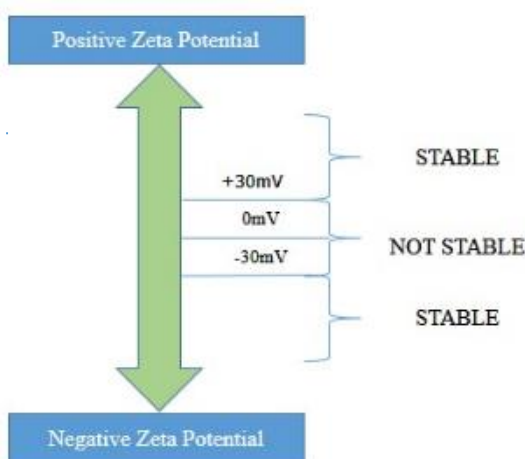


Fig 7.2.2.3 Zeta scale of the stability

7.6.4 Polydispersity Index

The polydispersity index is a measurement of the width of the molecular weight distribution (MWD). The MWD data analysis is the interpretation and comparison of various molecular distributions of polymers obtained experimentally.⁶⁸ Standard polydispersity index 0.1-0.5

7.6.5 Transmission Electron Microscopy (TEM)

The TEM imaging of the sample is done to observe the scanned images of the prepared phytosome under very high resolution 200000x to determine the particle size of the phytosome and entrapped drugs in the lipid.⁶⁹

7.7 *In-vitro* studies of the formulation

7.7.1 Antioxidant Activity

7.7.1.1 OH⁻ Scavenging Assay

OH⁻ radicals were originated from FeSO₄ and H₂O₂ and detected by their ability to hydroxylate salicylate. 3 ml of reaction mixtures were prepared by using 1 ml of FeSO₄ (1.5 mM), 0.7 ml H₂O₂ (6mM), 0.3 ml sodium salicylate (20mM) and 1 ml of different dilutions of the extract. Then, these mixtures were subjected for the incubation of 1 hour at 37°C. After incubation, absorbance of hydroxylated salicylate complex was recorded at 562 nm for different samples. Percentage of inhibition was calculated by using following formula.⁷⁰

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

Where,

A₁: Absorbance of extract with salicylate

A₂: Absorbance of sample without salicylate

A₀: Absorbance of the control

7.7.1.2 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assays

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 515nm. Percentage of inhibition was calculated by using following formula.⁷¹

$$\% \text{ Inhibition Percentage (I)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{control}}} \times 100$$

7.7.2 Anti-diabetic Activity

7.7.2.1 α -amylase inhibition assay

Starch iodine method was used for the determination of α -Amylase activity. 10 μ l of α -amylase solution (0.025 mg/ml) was mixed with 390 μ l of phosphate buffer containing different concentrations of extract. After incubation at 37 °C for 10 min, 100 μ l 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.⁷²

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of sample} - \text{Abs. of control})}{(\text{Abs. of blank} - \text{Abs. of control})} \times 100$$

Where, Absorbance of the sample (extract, α -amylase, starch), Absorbance of blank (no α -amylase), and Absorbance of control (no starch)

CHAPTER VIII

RESULT AND DISCUSSION

Table No. 8.1 Organoleptic character of the spinach

Sr. No	Parameters	Observation
1	Colour	Yellowish Green to dark Green
2	Odour	Characteristic
3	Taste	Sweet
4	Texture	Smooth

Table No. 8.2. Physicochemical Analysis of Spinach

Sr.No.	Physical Parameters	Results (%)			Mean
		Batch 1	Batch 2	Batch 3	
1	Foreign Matter (%)	NIL	–	–	NIL
2	Loss of Drying (%)	9.5	9.34	8.67	9.17
3	Ash Value (%)	30	28.23	30.02	29.41
4	Acid insoluble Ash (%)	6.5	5.72	5.94	6.05
5	Water Soluble Extractive Value (%)	59.2	53.45	52.51	55.05
6	Alcohol Soluble Extractive Value (%)	48	45	42.3	45.1

Standards of the physicochemical analysis of spinach were not found. However, results of the physicochemical parameters performed during the study as LOD (9.17 %), ash Value (29.41 %), acid insoluble ash (6.05%), water-soluble extractive value (55.05), Alcohol soluble extractive Value (45.1%) was found.

Table No. 8.3 Qualitative Analysis of spinach

Sr.no	Test	Chemical Tests	Result	Observation
1	Alkaloid	Mayer`s reagent	-Ve	NA
		Wagner`s reagent	-Ve	NA
		Dragendorff`s reagent	-Ve	NA
		Hager`s reagents	-Ve	NA
2	Saponin glycoside	Foam test	+Ve	Fome Appear
3	Reducing sugar	Benidict`s reagents	+Ve	Green colour
		Fehling`s test	+Ve	Brick red colour
4	Monosaccharaides	Barfoed`s Test	-Ve	NA
5	Amino acid	Ninhydrin Test	-Ve	NA
6	Steroids	Salkowski reaction	+Ve	Greenish yellow Florescence
7	Protein	Biuret test	+Ve	Voilet colour

+ve =Present,-ve =Absent

Qualitative analysis of the spinach indicates the presence of saponin glycoside, reducing sugar, monosaccharaides, steroid and proteins. These constituents are the main components of the good nutraceuticals.

Table No. 8.4 Nutritional value Analysis

Sr.no	Nutritional value	Results			Mean
1	Moister content (%)	2	1.94	2.03	1.99
2	Total crude fiber (%)	4.49	4.21	4.57	4.42
3	Protein (%)	0.057	0.054	0.047	0.052
4	Oil and fat (%)	0.7	0.74	0.71	0.71
5	Carbohydrate (%)	62.74	61.52	61.72	61.99
6	Vitamins A (µg)	27	26.56	27.02	26.86
7	Vitamin C (µg)	20	19.36	19.81	19.72

Nutritional Value analysis of spinach having Moisture content: 1.99, Total crude fiber: 4.42, Protein: 0.052, Oil and fat: 0.71, Carbohydrate: 61.99, vitamins: 26.86, vitamins: 19.72.

Table No. 8.5 Extraction of the Spinach

Sr.no	Wt. of leaves (gm)	Solvent used	Temperature (°C)	Duration (Hrs)	Wt. of extract obtained(gm)
1	100	Ethanol	50	72	0.12
2	100	Ethanol	50	72	0.11
3	100	Ethanol	50	72	0.13

Table no.13 Extraction of spinach in different batches

In this extraction, 100 gm. of fresh spinach was extracted with ethanol at 50°C for 72 hrs. All the batches gave the approximately same amount of the solid extract i.e. 0.12, 0.11, 0.13 after evaporation of ethanolic liquid media.

Table No. 8.6 Thin Layer Chromatography (T.L.C.) Analysis

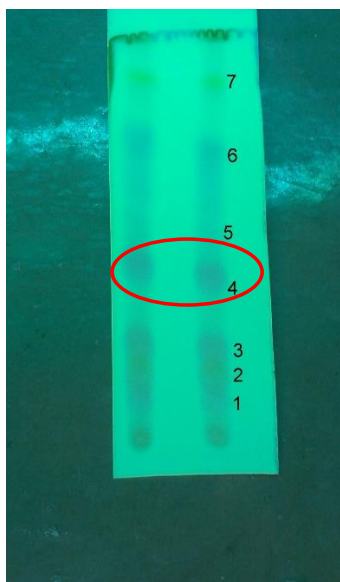


Fig: 8.6 TLC in Visible light

Mobile Phase: Chloroform: Isopropyl Alcohol: Acetic Acid (12:8:1)

Solvent Travel: 6.7

Sr No.	Found Spots	Rf value	Standard Rf
1	0.6	0.089	
2	0.9	0.134	
3	1.7	0.25	
4	2.6	0.38	0.38(Ref. ⁷⁵)
5	3.7	0.55	
6	4.6	0.68	
7	5.7	0.85	

Total 7 Rf were found in TLC study which revealed the presence of different compound in the extract. Rf 0.38 verify the having 20-hydroxyecdysone.⁷⁵

8.7. High Performance Thin Layer Chromatography (HPTLC) Analysis

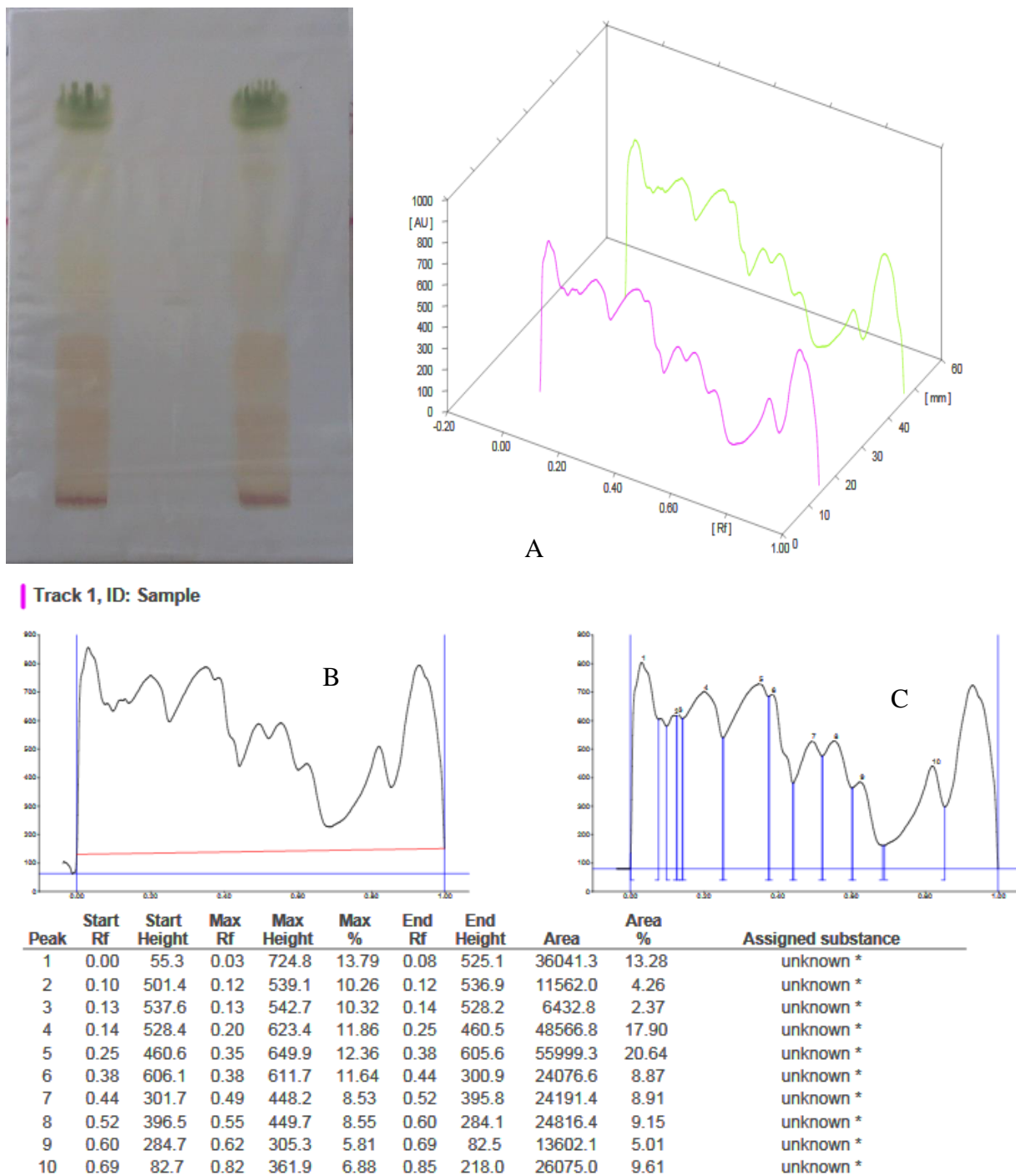


Fig 8.7 HPTLC Analysis of Spinach

Methanolic extract of spinach is analyzed by HPTLC using suitable solvent chloroform: Isopropyl Alcohol: Acetic Acid (12:8:1). There are 10 different compounds was detected with Rf (0.10, 0.13, 0.14, 0.25, 0.38, 0.44, 0.52, 0.60 and 0.69) among them peak no 6 with 0.38 was identified as 20-hydroxyecdysone.⁷⁵

8.8 UV. Spectrophotometer Analysis.

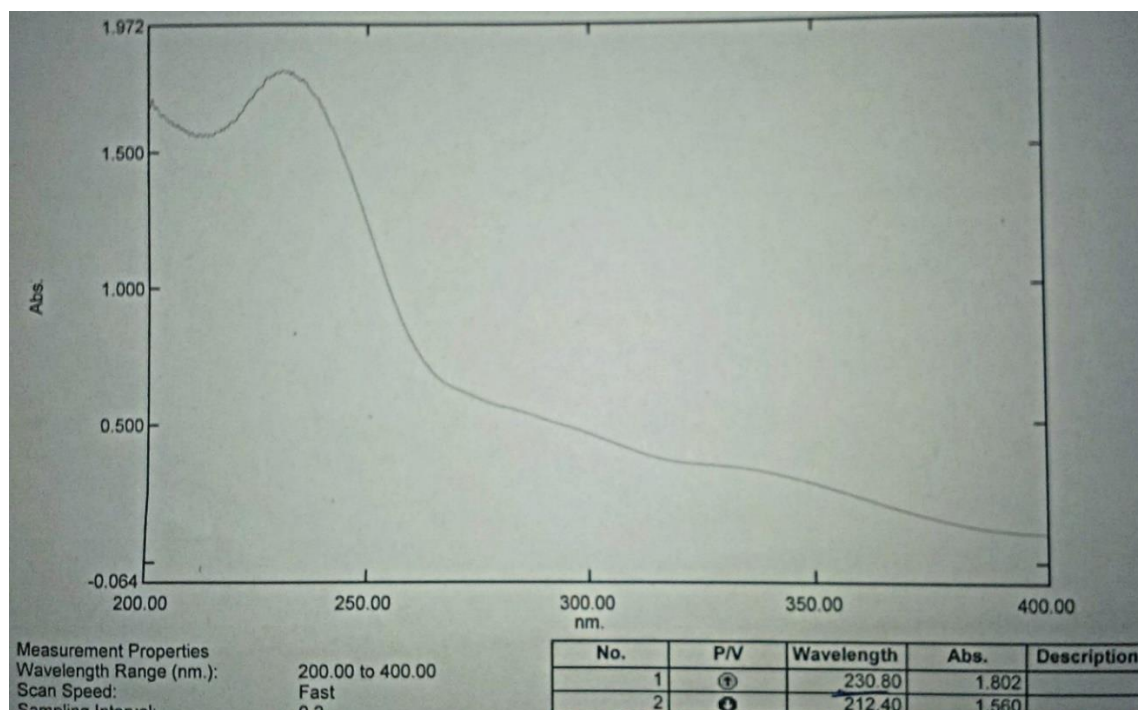


Fig. 8.8 (A) Analysis of λ_{\max} at 200-400nm

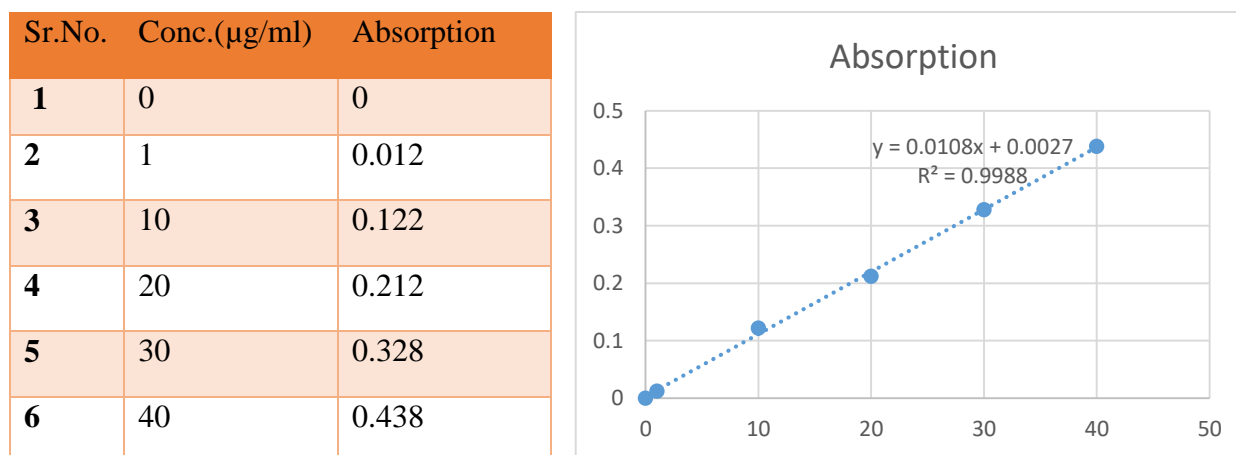
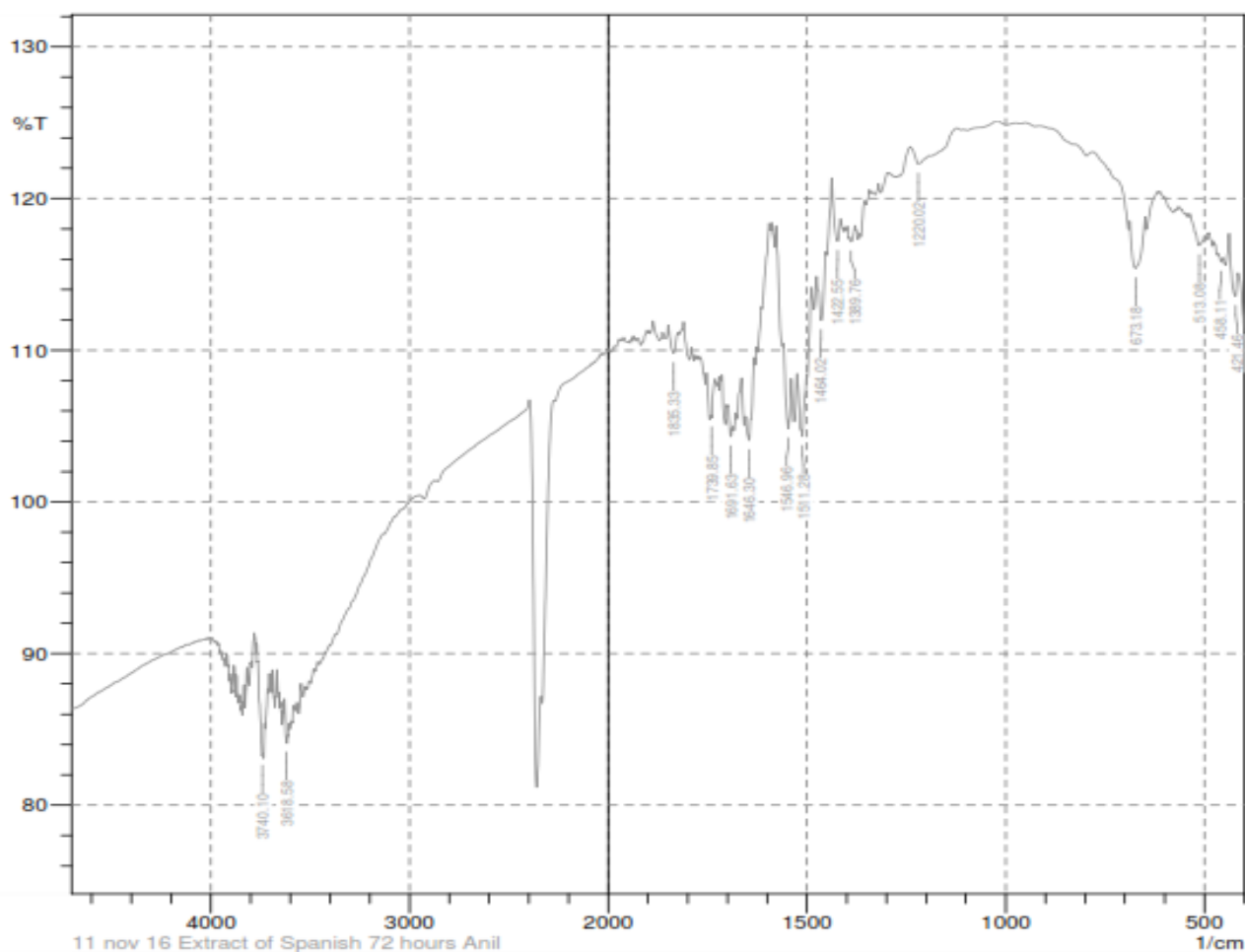


Fig. 8.8(B) Standard calibration curve

For the analysis of the extract different concentration was prepared (1 -40) mg/ml. Then λ_{\max} of the extract was determine by 1mg/ml by scanning from 200-400nm on Uv spectrophotometer (UV-1800, Shimzadu, Japan double beam spectrophotometer), shown in the fig.8.8 (A). To determine the linearity different concentration of the extract were analyzed at λ_{\max} 230nm and absorbance was recorded. The plot was prepared using concentration and respect to absorbance of the extract (8.8.B) and linear regression equation was obtained $Y=0.0108x+0.0027$, $R^2 =0.9988$, R^2 value is closed to 1 which on firm the linearity of the sample as per Beer`s Lambert`s law.

8.9 IR Spectrophotometer Analysis



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	421.46	113.556	2.21	437.86	415.67	-1.32	0.141
2	458.11	115.826	0.367	466.79	453.29	-0.875	0.007
3	513.08	116.907	0.245	521.76	511.15	-0.73	0.008
4	673.18	115.399	3.213	688.61	649.07	-2.616	0.32
5	1220.02	122.282	0.878	1240.27	1192.05	-4.283	0.071
6	1389.76	117.156	0.444	1392.65	1378.18	-1.014	0.017
7	1422.55	117.198	0.387	1424.48	1415.8	-0.613	0.009
8	1464.02	111.964	3.702	1475.59	1452.45	-1.283	0.18
9	1511.28	104.32	4.064	1524.78	1500.67	-0.631	0.213
10	1546.96	104.818	4.197	1559.5	1539.25	-0.577	0.204
11	1646.3	104.057	3.087	1654.98	1632.8	-0.528	0.173
12	1691.63	104.306	1.359	1697.41	1685.84	-0.242	0.034
13	1739.85	105.553	0.351	1740.81	1730.21	-0.315	-0.008
14	1835.33	109.795	1.602	1848.83	1823.76	-1.087	0.09
15	3618.58	84.123	0.25	3629.19	3617.61	0.805	0.021
16	3740.1	83.241	0.599	3763.24	3738.17	1.67	0.075

Peaks

1220.02 : O - H

1389.76 : C - O

1464.02 : C = C

1646.30 : C = O

1691.63 : C = O

Fig.8.9 IR Graph Representation

The extract was analyzed by IR spectrophotometer which showed various functional group i.e. 1220cm^{-1} (O-H phenolic), 1389cm^{-1} (C-O bond), 1464cm^{-1} (C=C bond), 1646cm^{-1} (C = O bond).

8.10 Preparation of Phytosome

Table No. 8.10 Different batches for the preparation of the phytosome loaded with lipid or drugs

Sr.No	Quantity of Soya lecithin	Quantity of cholesterol	Quantity of <i>Spinacia oleracea</i> Extract	Result
1	1part	1part	1part	Presence of vesicles
2	1part	2part	1part	Presence of vesicles with turbid
3	2part	1part	1part	Presence of vesicles phase separation
4	2part	2part	1part	Presence of vesicles

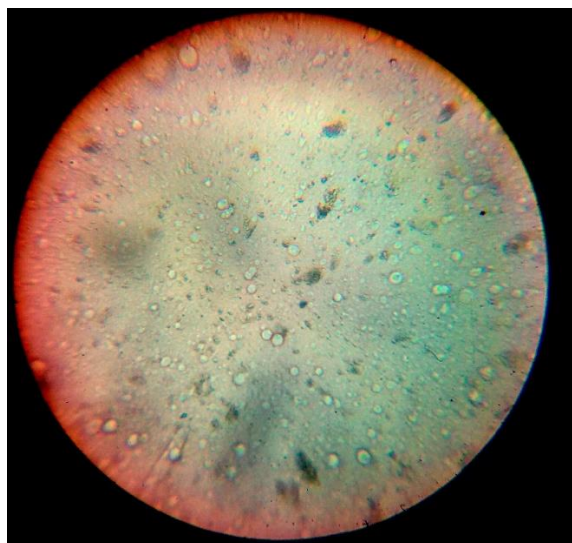


Fig.8.10.1 Btach 1



Fig.8.10.2 Batch 2

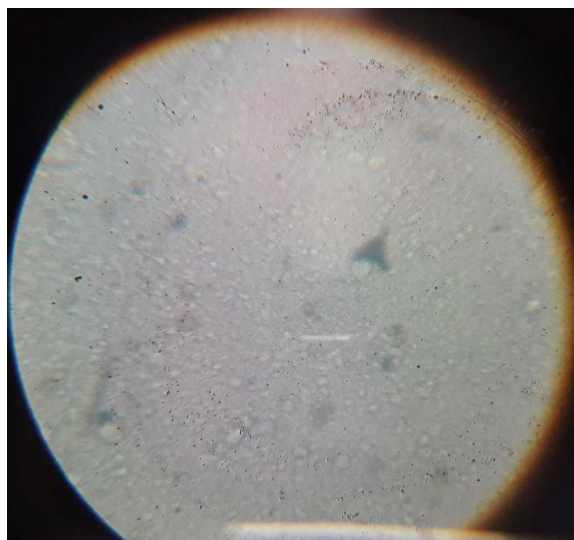


Fig.8.10.3 Batch 3



Fig.8.10.4 Batch 4

The phytosome loaded with extract was analyzed by the optical microscope which showed that all the batch contain vesicles at 45X, Shown in the fig 10.1,10.2, 10.3, 10.4. The Particle size of the phytosome is analyzed on the Beckman Coulter and which was 188.8nm.

8.11 Characterization of Phytosome

- Entrapment efficiency
- Particle Size
- Polydispersity index
- Zeta Potential
- TEM Analysis

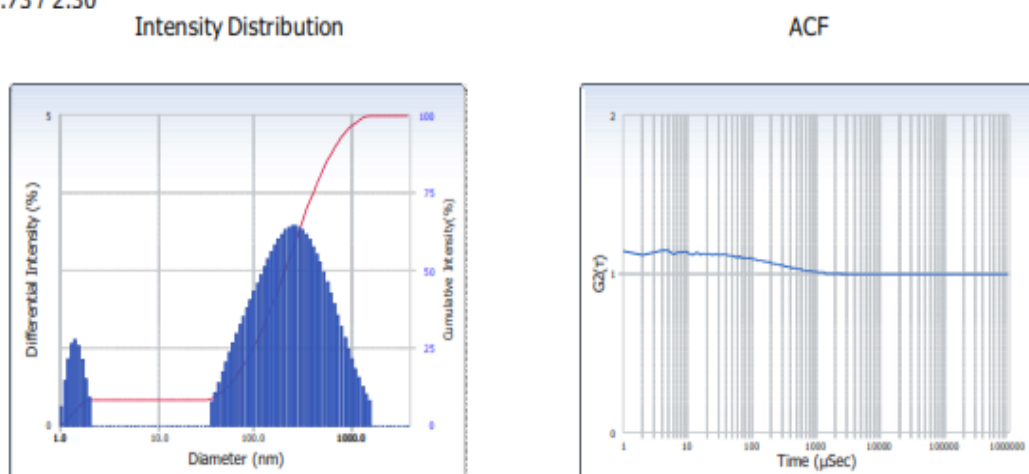
8.11.1 Entrapment efficiency

Entrapment Efficiency : 84%

Entrapment Efficiency is a most important parameter so, characterization of the phytosome entrapment efficiency (Refer sec.7.3.1) of extract was done by this evaluation we can identified that how much quantity of the drug entrapped in the phytosome vesicles. The phytosome entrapment efficiency of the extract was calculated up to 84% which showed good entrapment efficiency of the phytosome.

8.11.2. Particle Size Analysis

Version 3.73 / 2.30

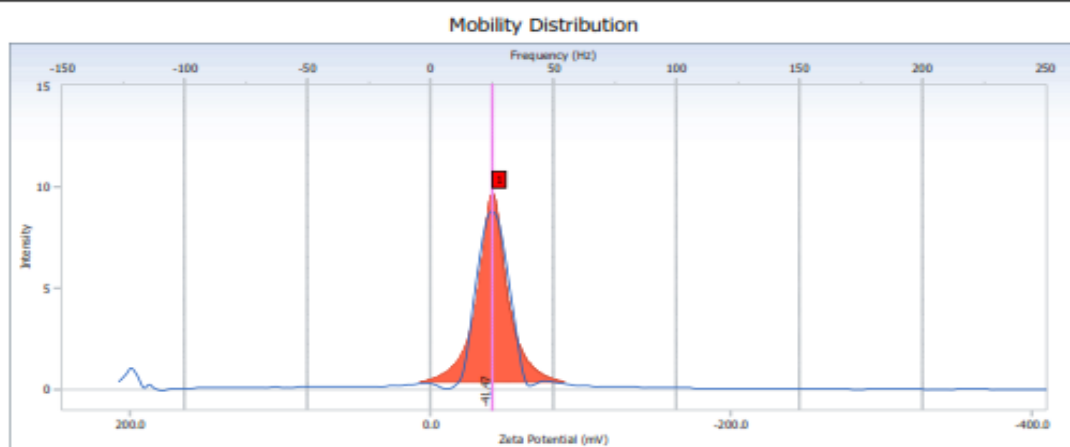
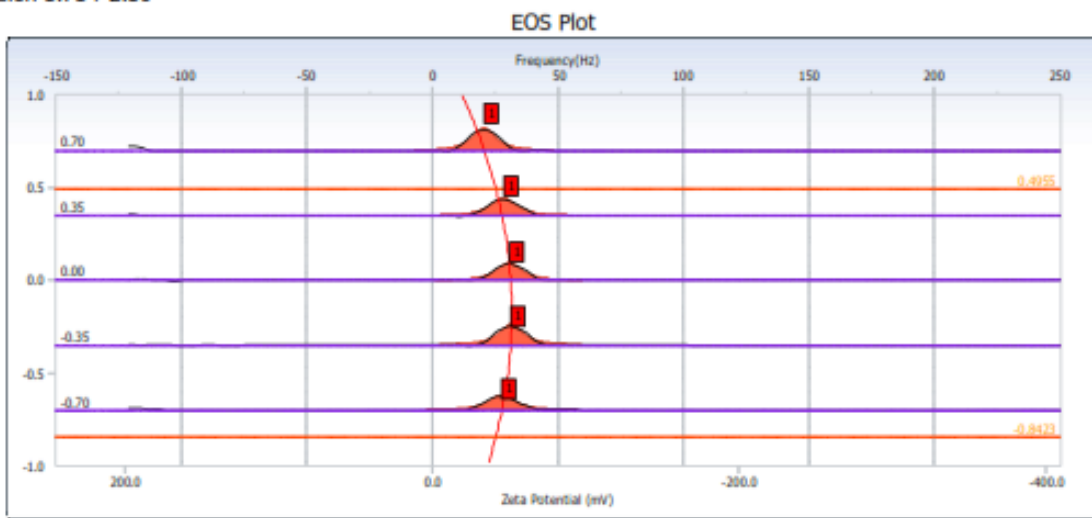


Distribution Results (Contin)			Cumulants Results		
Peak	Diameter (nm)	Std. Dev.	Diameter (d)	: 188.3	(nm)
1	1.4	0.3	Polydispersity Index (P.I.)	0.357	
2	349.7	304.8	Diffusion Const. (D)	: 2.626e-008	(cm ² /sec)
3	0.0	0.0	Measurement Condition		
4	0.0	0.0	Temperature	: 25.2	(°C)
5	0.0	0.0	Diluent Name	: WATER	
Average	320.4	307.3	Refractive Index	: 1.3328	
Residual :	2.687e-003	(O.K)	Viscosity	: 0.8838	(cP)
			Scattering Intensity	: 31146	(cps)
			Attenuator 1	: 30.38	(%)

The particle size of the phytosome is analyzed using the Beckman Coulter which standard average diameter of the particle was 320nm with polydispersity index 0.357. Polydispersity index is in the range of standard 0.1- 0.500.⁷³

8.11.3 Zeta Potential Analysis

Version 3.73 / 2.30



Measurement Results

Zeta Potential	: -41.47	(mV)	Doppler shift	: 25.34	(Hz)
Mobility	: -3.234e-004	(cm ² /Vs)	Base Frequency	: 121.0	(Hz)
Conductivity	: 0.6421	(mS/cm)	Conversion Equation	: Smoluchowski	

Zeta Potential of Cell			Diluent Properties		
Upper Surface	: -21.76	(mV)	Diluent Name	: WATER	
Lower Surface	: -5.52	(mV)	Temperature	: 25.0	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8878	(cP)
Avg. Electric Field	: -16.23	(V/cm)	Dielectric Constant	: 78.3	
Avg. Current	: -0.52	(mA)			

Phytosome was analyzed after 3 days of preparation to find out the zeta potential for the stability study of the phytosome. Spectra showed zeta potential -41.47mV for the preparation and it found above the standard range this range (+30 or -30 mV) which reflect its stability.⁷⁴

8.11.4 Transmission Electron Microscope (TEM) Analysis

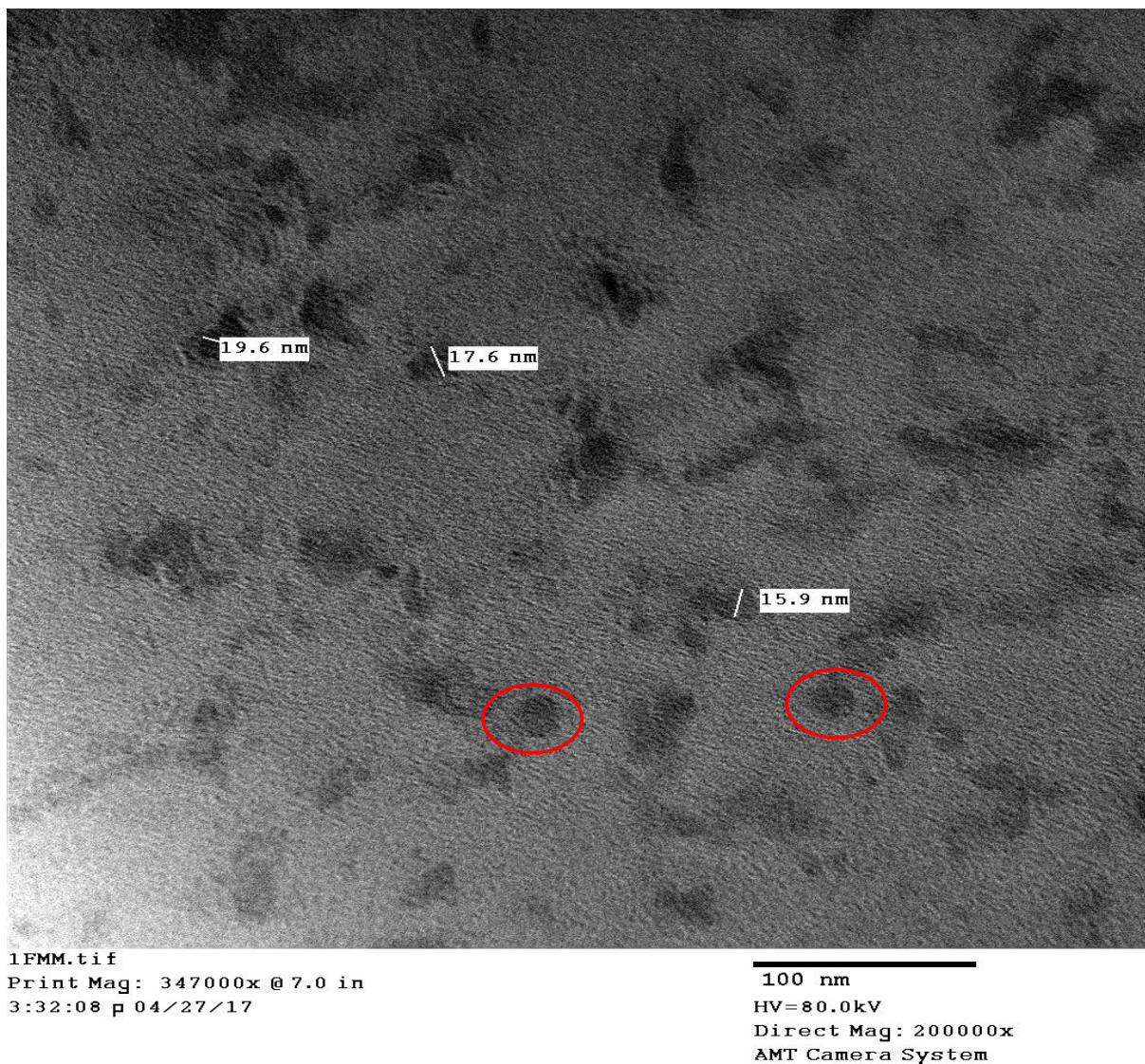
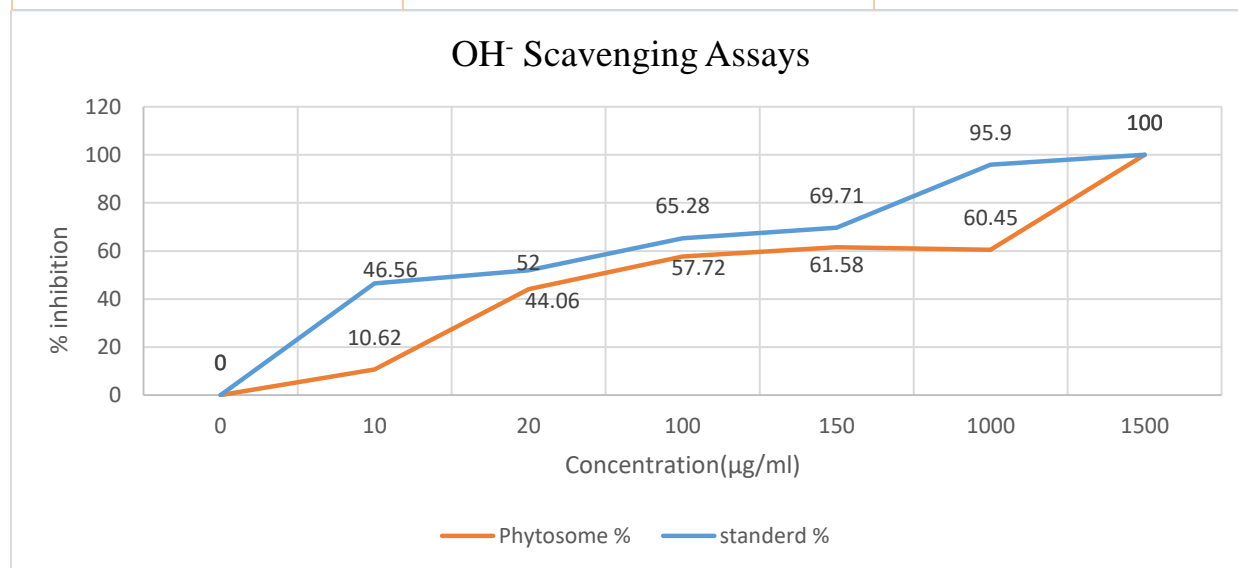


Fig 8.11.4 TEM Analysis

Transmission electron microscope of phytosome loaded with extract as showed in and this fig 8.11.4 at 200000x. The size of the particle was measured in the range of 15.9-19.6nm and Average size was 17.7nm. Picture also showed the loading of the drug encircled.

8.14. In-vitro study of the Phytosome loaded with Spinach**Table No. 8.14.1. Antioxidant Activity****a) OH⁻ Scavenging Assay**

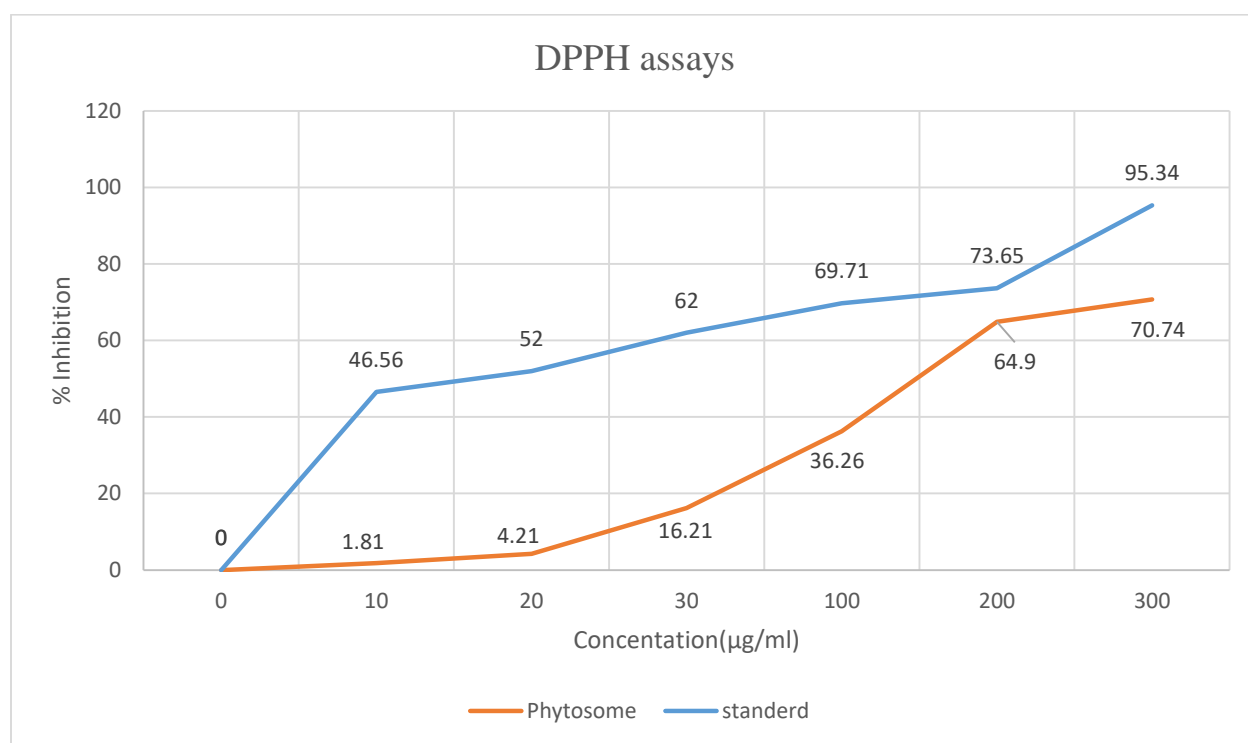
Concentration $\mu\text{g/ml}$	Ascorbic Acid	% Inhibition Formulation
0	0	0
10	46.56	10.62
20	52	44.06
100	65.28	57.72
150	69.71	61.58
1000	95.9	60.45
1500	100	100



Different concentration (10-1500 $\mu\text{g/ml}$) of standard antioxidant i.e. ascorbic acid and the phytosome loaded extract were prepared for anti-oxidant activity using OH⁻ Scavenging Assays. The result showed that phytosome was found comparable with ascorbic acid from concentration varying from 20 to 100 $\mu\text{g/ml}$ showing the inhibition approximately 50% - 60% respectively. Both standard and phytosome showed the 100% inhibition at 1500 $\mu\text{g/ml}$. IC₅₀ of the standard and formulation was calculated 3.28 $\mu\text{g/ml}$ and 3.98 $\mu\text{g/ml}$ respectively. It showed that phytosome was good antioxidant activity which is comparable with ascorbic acid.

b) DPPH Assays

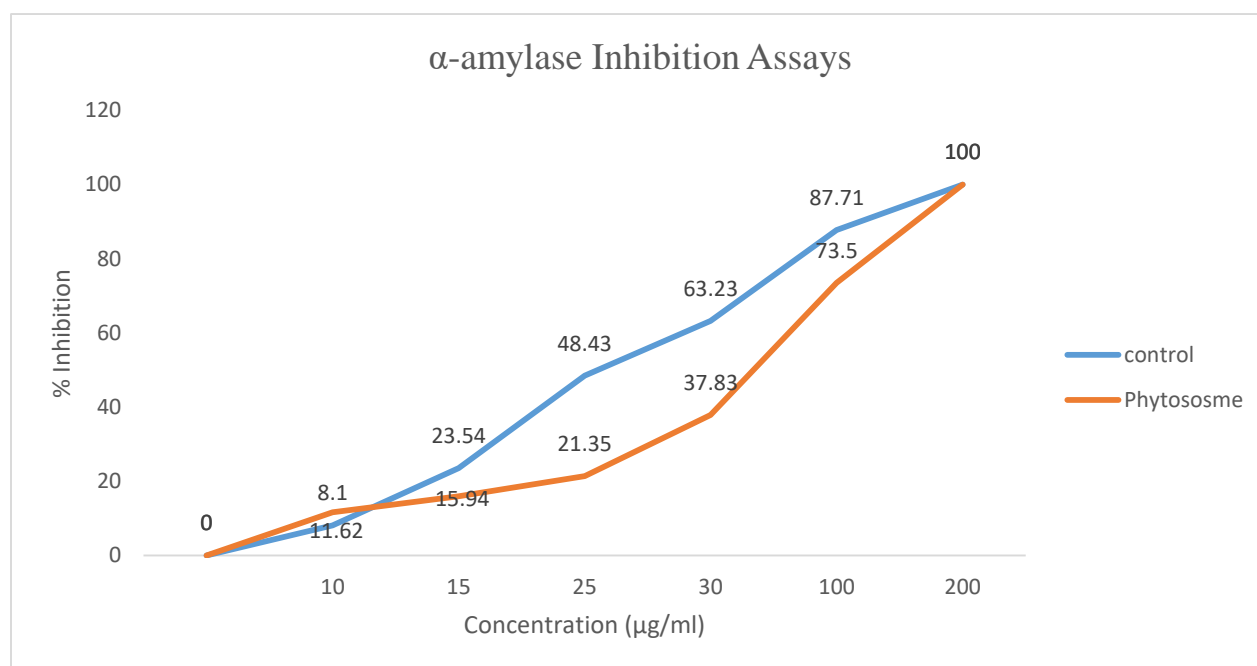
Concentration $\mu\text{g/ml}$	Ascorbic Acid	% Inhibition Formulation
0	0	0
10	46.56	1.81
20	52	4.21
30	62	16.21
100	69.71	36.26
200	73.65	64.9
300	95.34	70.74



Different concentration (10-300 $\mu\text{g/ml}$) of standard antioxidant i.e. ascorbic acid and the phytosome loaded extract were prepared for anti-oxidant activity using DPPH Scavenging Assays. The result showed that phytosome was found comparable only at 200 $\mu\text{g/ml}$ concentration with ascorbic acid. IC_{50} of the standard and formulation were calculated as 3.28 $\mu\text{g/ml}$ and 3.98 $\mu\text{g/ml}$ respectively. It showed that phytosome has moderate DPPH scavenging activity compared to ascorbic acid.

Table No.8.14.2. Anti-diabetic Activity**a) α -amylase inhibition assay**

Concentration $\mu\text{g/ml}$	Acarbose % Inhibition	Formulation % Inhibition
0	0	0
10	8.1	11.62
15	23.54	15.94
25	48.43	21.35
30	63.23	37.83
100	87.71	73.5
200	100	100



Different concentration (10-200 $\mu\text{g/ml}$) of standard anti-diabetic agent i.e. acarbose and the phytosome loaded extract were prepared for anti-diabetic activity using α -amylase inhibition assays. The result showed that phytosome was found comparable with acarbose from concentration varying 10 to 100 $\mu\text{g/ml}$ with the inhibition approximately 40% - 80%. Both standard and phytosome showed the 100% inhibition at 200 $\mu\text{g/ml}$ concentration. IC_{50} of the standard and formulation was calculated 3.28 $\mu\text{g/ml}$ and 4.98 $\mu\text{g/ml}$ respectively. It showed that phytosome was good anti-diabetic activity comparable with acarbose.

CHAPTER IX

CONCLUSION AND FUTURE SCOPE

The increasing demand leads to the new scope of research and drug development in the field of nutraceuticals. Besides, Ayurveda has great potential to provide established and time-tested drugs to develop potent nutraceuticals. Ayurveda mentioned the leafy vegetables are the good source of nutrients. Whereas, it has potential to be a potent nutraceutical in the form of novel delivery system to enhance the stability, solubility, bioavailability, safety, and the efficacy of the drugs conceding that we have aimed to develop the novel drug delivery of the spinach.

The development process includes the procurement of the spinach, authentication, standardization, of the spinach. All the results of the standardization were done first time because no standard is available in the official compendiums. Extraction of the spinach is done by the hot extraction with a suitable polar solvent. The obtained extract was characterized by the TLC, HPTLC, IR, and UV. However, characterizes extract was concluded to manufacture the different batch of formulation development in the form of novel drug delivery. That optimized batch is further characterized with the microscope on 45X, particle size, Zeta size and PDI analysis were done on Beckman Coulter which has shown the size of prepared phytosome to be 320nm and PDI is 0.357. The entrapment efficacy of the lipid vesicle is 84%. The TEM images obtained confirmed the preparation of the phytosome prepared. IC_{50} of antioxidant (OH^- and DPPH) anti-diabetic activities were (3.98 μ g/ml) and (4.98 μ g/ml), respectively formulation was found effective as standard.

Future scope: Stability study and *in-vivo* study of the phytosome are required to establish it as an effective nutraceutical.

CHAPTER X

REFERENCES

- 1 Ghosh D. The Nutraceutical potential of Ayurvedic medicines, Nutrient insight 2014:51-55
- 2 Chandaliya D. K, chandaliya. S. et.al. Clinical Consideration of 'Pathya kalpana' (Ayurvedic dietetics), International Ayurvedic Medical Journal 2015; Volume 3; 4:1240-1248
- 3 Agnivesh, Charak, Dridhabala, Charak Samhita, SutraSthan ,Deerghajeebiteeyadhyay, 1/24, edited by dr. Gorakh Nathchaturvedi, Chaukhamba orientaila, 2003: 8
- 4 Agnivesh, Charak, Dridhabala Charak Samhita, Chikasta asthan, Rasayana aadhy chapter 1, edited by Prof. Priyavrat Sharma vol 2 ,chaukhamba orientaila 8th edition 2007:3-34
- 5 Shusurat samhita, Chikasta asthan, 27th aadhy part 1, edited by kaviraj ambikaduttashastri, 2005:120-123
- 6 Sharma V, Chaudhary A. K. Concept of Dhatu Siddhartha (theory formation and differentiation) and Rasayana; probable predecessor of stem cell therapy, AYU2016, vol 35, Issue 3; 231-236
- 7 Agnivesh, Charak, Dridhabala , Charak Samhita. Chikasta sthan. Rsayana aadhy 1/3 edited by Prof. Priyavrat Sharma vol2, chaukhamba orientaila 8th edition 2007;13
- 8 Shusurat Samhita, Chikasta Sthana. 27th aadhy part 1, edited by kaviraj ambikaduttashastri, chaukhamba orientaila ,2005: 121
- 9 Shusurat Samhita, Chikasta Sthana. 45th aadhy part 1, edited by kaviraj ambikaduttashastri, chaukhamba orientaila ,2005:167-227
- 10 Agnivesh, Charak, Dridhabala, Charak Samhita, Charak Samhita, Sutra Stha, Deerghajeebiteeyadhyay, 28/45, edited by dr. Gorakh Nathchaturvedi, Chaukhamba orientaila 2003;8:28/45
- 11 Shahidi, F. et. al. Nutraceuticals, functional foods and dietary supplements in health and disease. Journal of Food and Drug Analysis 2012; vol 20, Issue1:226–230.
- 12 Chauhan B. et. al; Current concepts and prospects of herbal nutraceutical: A review; Journal of Advanced Pharmaceutical Technology & Research 2013; Vol 4 , Issue 1:4-8
- 13 <http://www.newhope.com/supply-news-amp-analysis/indian-nutraceuticals-market-could-double> (Date 27 Nov 2016)
- 14 <http://www.nutraceuticalsworld.com/> (Date 25 Nov 2016)

- 15 Chauhan B.et.al; Current concepts and prospects of herbal nutraceutical: A review; Journal of Advanced Pharmaceutical Technology & Research 2013; Vol 4 , Issue 1:4-8
- 16 Aklakur Md.et.al., Nano Delivery: An Emerging Avenue for Nutraceuticals and Drug Delivery, Critical Reviews in Food Science and Nutrition,2015 DOI: 10.1080/10408398.2013.839543
- 17 <http://plants.usda.gov/core/profile?symbol=spol> (14 Oct-15)
- 18 <http://www.sciname.info/Default.asp?SciName=Spinacia%20oleracea&Option=BeginWith%20> (15 Oct-2015)
- 19 Parmod C.et.al, Phytochemical investigation of *spinacia oleracea*: An important leafy vegetable used in Indian diet, Central European Journal of Experimental Biology 2015;Vol 4 ,Issue-1:1-4
- 20 <http://www.almanac.com/plant/spinach> (15 Oct -2016)
- 21 <https://www.plantvillage.org/en/topics/spinach> (16 Oct -2016)
- 22 <http://www.newworldencyclopedia.org/entry/Spinach> (16 Oct-2016)
- 23 Otarik.v.et.al. Spinacia oleracea Linn: a pharmacognostic and pharmacological overview, International journals of research and pharmacology 2010;Vol 1,Issue-1: 78-84
- 24 Agnivesh,Charak, Dridhabala ,Charak Samhita, Sutra Sthan. Aanapanvadiyadhan, Vol 1/100, edited by dr. Gorakh Nathchaturvedi, Chaukhamba orientaila 2003; 512
- 25 Shusurat Samhita, Sutra Sthana.46th aadhya part 1, edited by kaviraj ambikaduttashastri, chaukhamba orientaila ,2005; 205
- 26 Astangsanghardya, sutrasthan, annashuroopvigyanayo, edited by Dr. Ravi Dutta Tripathi, Chaukhamba orientaila 2003; 139
- 27 Shankar Nighantu, dutya vaga, edited by R.J. Pandit Shankar Dutta Gauda, Chaukhamba orientaila 2002,drug no-429,pp.156
- 28 Bhavprakesh nighantu, vol 1 Atha shak varga, edited by Dr. G.S. Pandey, Chaukhamba orientaila 2004 pp. 668
- 29 Sisodia R. et.al. Spinacia oleracea modulates radiation –induced biochemical changes in mice testis, Indian journal of pharmaceutical science, 2008 ;321-326
- 30 Grossman S. et.al. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions, Photochemistry 2001;vol 58: 143–152
- 31 Z. Yosefi , R. Tabaraki , H. A. Asadi Gharneh et.al. Variation in Antioxidant Activity, Total

- Phenolic, and Nitrate in Spinach, International Journal of Vegetable Science 2014; 16 : 233-242
- 32 Ang-Heui Ko, Jae-Hee Park, So-Yun Kim et.al. Antioxidant Effects of Spinach (*Spinacia oleracea* L.) Supplementation in Hyperlipidemic Rats, Prev. Nutr. Food Sci. 2014;vol 19, issue1:19-26
- 33 Abdul D A, Majeed S N, Ameen B H. Antioxidant activity, total phenolic content and antimicrobial activity of two medicinal plants from Sulaimani City, Iraqi Kurdistan Region, Advances in Life Science and Technology 2014;vol 8 : 65-71
34. Mizushima Y, et al. Effect of glycolipid from spinach on mammalian DNA polymerases. Biochem Pharmacology, 2003; Vol 65 : 259-267.
- 35 Gupta RS, Singh D.et.al., Amelioration of CCl₄-induced hepatosuppression by *Spinacia oleracea* L. leaves in wistar albino rats. Pharmacology online 2006 ;Vol 3:267-278
- 36 R. Eden harder et.al. Inhibition of clastogenicity of benzo[a]pyrene and of its trans-7,8-dihydrodiol in mice in vivo by fruits, vegetables, and flavonoids, Mutation Research 2003;Vol 537 : 169-18
- 37 Naoki M. et.al. Anti-Tumor Effect of Orally Administered Spinach Glycolipid Fraction on Implanted Cancer Cells, Colon-26, in Mice, Lipids, 2008; Vol 43: 741–748,
- 38 Asija R., Kumawat R., Sharma D, Sagar P. Evaluation of Anti – cancer Activity of Ethanolic Extract of *Spinacia oleracea* by High Throughput Screening, Int. J. Pharm. Sci. Rev. Res 2015;vol 33,2,:225-227
- 39 Das S. et.al. CNS depressive role of aqueous extract of *Spinacia oleracea*. L. leaves in adult male albino rats, Indian journal of experimental biology.,2008,Vol 46 ,pp. 185-190
- 40 Patil U. K. in-vitro Antihelmintic Activity of Leaves of Spinach *oleraceae* Linn., International Journal of Toxicological and Pharmacological Research 2009; Volume 1, Issue 1: 21 - 23
- 41 Pandya S.,Patel K. Phytosome: Technical Revolution In Phytomedicine, International Journal of Pharm Tech Research 2010;Vol 2.Issue No. 1: 627-631
- 42 Jain N., Gupta B.P., Thakur N., Jain R. et.al. Phytosome :A novel Drug Delivery System for Herbal Medicine, International Journal of Pharmaceutical Sciences and Drug Research 2010; Vol 2,Issue 4 : 224-228

- 43 Parris M. Kidd. Bioavailability and activity of phytosome complex from botanical polyphenols: The silymarin, Curcumin, Green Tea, and Grape Seed extract, *Alternative Medicine review* 2009; Vol 14: 226-246
- 44 [http://www.indena.com/phytosome/\(4-Dec-2016\)](http://www.indena.com/phytosome/(4-Dec-2016))
- 45 Pandey S, Patel K. Phytosomes: technical revolution in phytomedicine, *Int. J. Pharm Tech Res* 2010; Vol 2 : 627-31.
- 46 Anupama Singh, Vikas Anand Saharan et.al. Phytosome: Drug Delivery System for Polyphenolic Phytoconstituents, *Indian Journal of Pharmaceutical Sciences* 2011; vol 7, issue-4 : 209-219
- 47 Jain N, Gupta B P, Thakur N, Jain R et.al. Phytosome : A novel Drug Delivery System for Herbal Medicine, *International Journal of Pharmaceutical Sciences and Drug Research* 2010, Vol 2, Issue 4 : 224-228
- 48 Nayyer Karimi, Babak Ghanbarzadeh et.al. Phytosome and Liposome: The Beneficial Encapsulation Systems in Drug Delivery and Food Application, *Applied food biotechnology* 2015; vol 2 issue-317-27
- 49 <http://nutritiondata.self.com/facts/vegetables-and-vegetable-products/2626/2> (Date 28-nov 2016)
- 50 The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed. 1999, part I, Vol.-II, Apx-3 Page no 190
- 51 The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed. 1999, part I, Vol.-II, Apx-3 Page no 191
- 52 T. Prashant, Bimlesh Kumar et.al. Phytochemical screening and Extraction : A Review, *International Pharmaceutica Science* 2011; 1: 98-106
- 53 Anayo Joeshep"raku, Stanely Chukwudozie onuoha et.al. Nutritional and Anti-Nutritional Quantification Assessment of Cymbopogon citratus Leaf, *Pharmacology and Pharmacy* 2015; 6: 401-410
- 54 Anonymous, Protocol for testing the Ayurvedic, siddha and unani medicines, Published by Government of India, Department of Ayush, Pharmacopoeial laboratory of India medicines. page No. 57

- 56 Obadoni B.O, Ochuko P.O. Phytochemical studies and comparative efficacy of the crude extract of some homeostic plants in edo and delta states of Nigeria, Global Journal pure Applied science 2001; 8: 203-208
- 57 AOAC Official Method of Analysis of Association of Analytical chemists International 2000 ;17th edition, Horowitz, maryland
- 58 Hussian I ,Saleem M, Iqbal Y. et.al. Comparision of vitamin C contents in commercial tea branda and fresh tea leaves, journal of chemical society of Pakistan 2006; 28: 971-973
- 59 Robert J Grebenko, Perry v. Ripa et.al. Occurrence and level of ecdysteroids in spinach, lipids 1991; vol.26, 8: 666-668
- 60 The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed.1999, part I, Vol.-II, Apx-3 Page no195
- 61 Reich E, Schibli A. Stationary Phases for Planar Separations - Plates for Modern TLC. LC GC. 2005; 23:58-69
- 62 Ashwini S Dhase, Shweta S Saboo et.al. Preparation and Evaluation of Phytosomes Containing Methanolic Extract of Leaves of Aegle Marmelos (Bael), International Journal of PharmTech Research 2015; Vol.8: 231-240
- 63 Babak Ghanbarzadeh, Afshin Babazadeh et.al. Nano-phytosome as a potential food-grade delivery system, Food Bioscience 2016; 15:126-135
- 64 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
65. <http://www.particulatesystems.com/Products/NanoPlus-DLS-/Principle-of-Zeta-PotentialMeasurement.aspx> 14th April 2017
- 66 <http://www.silver-colloids.com/Tutorials/Intro/pcs12A.html> 14th April 2017
- 67 Jose Antiniob Alves, Joao Baptista Bald. The Behavior of Zeta potential of silica suspension, New journal of glass and caramics 2014;14: 29-37
- 68 M.rogosic, H J mencer et.al. Polydispersity index and molecular weight distributions of polymers, eup.polym.j.1996; vol.32, 11:1337-1344
- 69 Felix Borrnet..Thought about next generation (S)TEM instrument in science, micrin 2015; 90: 1-5

- 70 Hua Wang,Xiang Dong Gao et.al. In vitro and in Vivo antioxidant activity of aqueous extract from choerospondias axillaries fruit, Food Chemistry 2008; 106: 888-895
- 71 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 2009; 114: 889-897
- 72 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
- 73 Manasi M. Chogale, Vinod N. Ghodake. Performance Parameters and Characterizations of Nanocrystals: A Brief Review, Pharmaceutics 2016; 8:26
- 74 https://caliscc.org/images/presentations/Morante_Zeta_Potential.pdf
- 75 Atul N. Jadhav,chidananda swamy Rumallaet.al. HPTLC method for determination of 20-Hydroecdysone in *Sida rhombifolia* L. and dietary supplements, chromatographia 2007;66:9-10

CHAPTER XI

Appendixes



TOPIC APPROVAL PERFORMA

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : 5303H::B. Pharmacy (Ayurveda) – M. Pharmacy (Ayurveda)(Dual Degree)

COURSE CODE : APH023

REGULAR/BACKLOG : Regular

GROUP NUMBER : PHRRGD0036

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	Anil Kumar Sah	11105847	2011	Y1553	9646531508

SPECIALIZATION AREA : Ayurvedic Pharmacy

Supervisor Signature: _____

PROPOSED TOPIC : Pharmaceutical development and evaluation of oral dosage form prepared from spinach

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	7.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.50
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.	7.50
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.	7.50


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): Yes

Final Topic Approved by PAC: Pharmaceutical development and evaluation of oral dosage form prepared from spinach

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. 1334 Bot. & Env. Sc.
Dated 24/11/2016

To Whom It May Concern

The plant specimen(s) brought by Mr. Anil Kumar Sah
Regn. No. _____ student of M. Pharmacy (Ayurveda) L.P.U, Phagwara (P)
1105847 belongs to the following species.

1. ✓ Spinacia oleracea (Spinach)
2. Amaranthaceae
3. _____

Signature of Student Anil
Herbarium Assistant _____
Teachers Incharge Re

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

Phone: +91 183 2451048, PABX: 0183-2258802-09, 2450601-14 Extn. 3193, Fax : 0183-2258819-20 and 2255711
Website: <http://www.gndu-dobes.org>; e-mail: gndu_botanical@hotmail.com